THE EFFECTS OF VOLUNTARY EXERCISE ON A RODENT MODEL OF BINGE EATING

by
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Abstract:

Binge eating disorder (BED) is an eating disorder involving repeated, intermittent over consumptions of food in brief periods of time, usually with no compensatory behaviors. Unfortunately, there has been limited progress in the development of treatment strategies, partially due to the fact that the neurological and physiological consequences of repeated bingeing are not clearly understood and cannot readily be studied in humans. Thus, in order to investigate behavioral and neurological pathways affected by BED, rodent models mimicking BED symptoms can be used. Some models use cycles of food restriction and refeeding (R/R), while others use stress to induce bingeing. There are also different types of highly palatable foods that have been used, including vegetable shortening, sucrose, and cookies.

Due to the fact that weight issues and physical inactivity can be central issues in BED, exercise may be a useful treatment. To date, no studies have been published examining the effects of exercise on binge-eating in rats. It has been shown that running wheel activity (RWA) can decrease consumption of a daily high-fat diet (HFD), but the effects of RWA on sporadic, intermittent consumption of a HFD (e.g. bingeing) are still unknown. In the studies presented here, we hypothesized that RWA could reduce binge-eating in rats. Rats were given sporadic (3 times/wk) limited (1hr) access to a HF food (Crisco), in addition to continuously available chow. Crisco was available every Mon, Wed, and Fri for 1hr before dark onset. We designed the experiments to assess whether RWA would reduce established binge-eating, as well as assess whether prior RWA would prevent binge-eating from developing.

We found that RWA significantly decreased Crisco intake, but prior RW access did not prevent bingeing. While the appetite hormones were expressed in the appropriate directions, bingeing rats were not modifying their intake, and thus another brain pathway may be interfering. There was evidence that the brain reward pathways of both bingeing and running rats were activated, and may be overriding the appetite hormones. Overall, access to a RW and the resulting activity significantly reduced binge-eating and modulated the effects of bingeing on brain appetite and reward systems.

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General Running Wheel Activity and Food Intake

Exercise has numerous well-known benefits to overall health (39, 40), as well as specific neural and cognitive effects (41), in both humans and laboratory animals. In this section, we will focus on how a running wheel can have various direct and indirect consequences on rodent energy balance. Here, energy balance is characterized as the biological homeostasis of energy in an organism, encompassing energy intake, storage, efficiency, and internal and external work produced.

Wheel-running behavior in rodents follows a fairly consistent pattern, increasing for a couple weeks until the total distance covered in a given period of time plateaus (52), with a concurrent increase in food intake that is presumed to occur in order to meet the increased energy demands (42, 43, 44, 45). Then, over time with ageing, the total running duration decreases (52). In most experiments, a running wheel is introduced in order to examine the effects of exercise as opposed to assess physical activity levels in general (46, 47). In fact, the physiological consequences of running wheel activity in rodents closely parallel the results of exercise in humans, including increased muscle insulin sensitivity, increased skeletal muscle citrate synthase, and decreased visceral fat relative to sedentary rats (48, 49).

Running wheels have the potential to influence several aspects of energy balance aside from simply the direct effects on energy expenditure. In rodents, females run to a

greater extent than males, often running approximately 10,000 revolutions per day while male rats only run approximately half as much (217). In female rats over their estrous cycle, one observes an advance in the onset of running and increases in running wheel activity occurring on the morning of estrus, which also coincides with decreased energy intake (7, 181). This effect is often species dependent, which may be influenced by high circulating concentrations of estradiol (50, 51). Experiments by Eckel et al. reveal that access to running wheels decreased dark meal frequency, increased dark-meal size, produced progressively larger meals through the dark, and increased 24-h water intake (182). Access to wheels, however, did not affect either 24-h food intake or body weight. During estrus, 24-h food intake, dark meal size, and body weight were reduced in both rats with and without wheels, whereas dark meal frequency was increased only in rats without wheels.

Variations in the amount of food available can affect physical activity, both with and without access to a running wheel. Generally, acute starvation increases overall physical activity over the short term in many species, which is thought to be a foraging response (54, 55). In contrast, long-term starvation decreases levels of physical activity (56, 57), presumably to conserve energy. However, during periods of starvation when there is a complete absence of food, the effects are different than during periods of caloric restriction, wherein rodents are fed a reduced number of calories each day, most commonly 60–70% of pre-restriction levels. Caloric restriction suppresses physical activity levels in rats (57). This is typically interpreted as an effort to conserve energy in the face of decreased food availability.

Thus, several factors interact in order to determine the degree to which food availability alters general, spontaneous activity in rodents. These include species, strain or genetic background, sex, phase of the estrous cycle, ambient temperature, the type and magnitude of food restriction, time of day, and of course the presence of a running wheel. Though innate general cage activity levels appear to be roughly correlated with running wheel activity levels (67, 68), it is clear that the two are not equivalent. It is also apparent that running wheel activity does not accurately represent general physical activity in rodents during caloric restriction, and that the presence of the wheel has profound effects on energy balance, especially in rats.

Exercise and Reward

Of particular interest lately is the importance of the brain reward system activation in relation to energy balance regulation, particularly as it relates to appetite (69, 70). These same brain reward systems play a role in running wheel behavior. In fact, the act of wheel running itself can become a self-perpetuating behavior that has the capacity to reach obsessive levels. It has been shown that rats will lever-press for access to running wheels (71, 72, 73, 74, 75), similar to their behavior in drug self-administration paradigms, which indicates that wheel running is also rewarding. This rewarding aspect of voluntary wheel running suggests that neural pathways that regulate the reward experienced after drugs of abuse may also be activated by wheel running. This idea is supported by the finding that rats that exhibit the highest levels of lever-pressing for running wheel access also exhibit the highest levels of running wheel activity (73), suggesting that those rats that receive the most

reward from wheel running are also willing to work the hardest for it. Additionally, this behavior suggests that animals are willing to engage in wheel-running behavior in a way that parallels humans' willingness to engage in drug use despite dangerous consequences (79).

Similar to other reinforcers, the running wheel can be used in hedonic substitution as an alternate reinforcer. For example, running wheel activity increases when mice are denied access to ethanol (80), and running wheel activity can serve as an alternative source of motivation, replacing drugs of abuse such as cocaine (81) because voluntary running wheel activity can substantially decrease intravenous self-administration of cocaine in rodents (82, 81). Wheel running also decreases oral consumption of amphetamine in rats (83). However, more relevant to energy balance, studies demonstrate that wheel running also decreases motivation for natural reinforcers such as food (73) and sucrose solutions (84). Conversely, food deprivation increases lever pressing for both cocaine and access to running wheels (85, 86). Therefore, in addition to directly impacting metabolism through exercise and its downstream impact on energy expenditure and appetite, wheel running may also alter energy balance via indirect means by modulating the rewarding and reinforcing aspects of food.

The Effects of Exercise on Diet Choice

In rodents, acute running wheel activity decreases food intake and body weight for a number of days (75). When the running wheel access is provided over a longer duration, food intake is increased to account for the increased energy expenditure but the body weight remains lower than that of sedentary controls (90). One potential mechanism that could

contribute to body weight control with exercise is through altering dietary preferences. Rats introduced to a sucrose solution and running wheel simultaneously engage in running wheel activity but avoid consuming the sweet solution (84). When solid palatable food is provided (e.g. high fat (HF) diet) wheel running can significantly reduce HF intake and thus decrease the preference for a previously preferred HF diet in a two-diet (standard chow vs. HF) choice feeding schedule (91, 92).

Sex differences have also been revealed in running wheel induced changes in palatable diet preference in rats. When running wheel access and a novel palatable HF or high sucrose (HS) diet were provided simultaneously, male rats showed persistent avoidance to the palatable diets during as well as after the running wheel access had ended (93). In contrast, female rats avoided the palatable diets for a few days but then gradually increased intake of the palatable diets and ended up preferring them before the running access had stopped. In another schedule when wheel running occurred for a few days before the access to a novel HF diet, both male and female rats initially showed hyperphagic responses to the HF diet. However, HF diet intakes of the male rats gradually declined and they ended up showing no preference between the HF and standard chow diets. Conversely, the female rats maintained high intake and preference for the HF diet throughout the running period. Finally, both males and females that had previously experienced simultaneous wheel running and HF diet consumption decreased their HF diet preference when the two stimuli were provided again after a period without access to them.

Liang et al. examined whether running wheel access and the resulting activity alter intake of a preferred HF diet in rats on a continuous ad lib access feeding schedule (93). Prior to wheel running access, rats developed persistent preferences to the HF diet and consumed significantly more HF diet than the high carbohydrate chow diet. As soon as running wheel access was allowed, the rats ran and reduced their HF diet intake significantly. Consequently, the HF diet was less preferred by the wheel running (WR) rats and resulted in a HF diet preference ratio less than that of the sedentary (SED) controls. Although paired- fed rats consumed the same amount of HF and chow diets as the WR rats, they had reduced expression of Proenkephalin-A (PENK) in the ventral tegmental area (VTA) and nucleus accumbens (NAc) and reduced expression of the dopamine-2 receptor (D2R) in the NAc, but showed increased expression of dopamine transporter (DAT) in the VTA. Additionally, the gene expression profiles of the WR and SED rats were similar; both had elevated PENK expression in the VTA, despite differences in HF and chow diet consumption between the two groups. Overall, the results indicate that running can reduce exogenous opioid agonist stimulated or daily non-stimulated HF diet intakes. Furthermore, running may maintain the gene expression profile of PENK and dopamine (DA) receptors at levels similar to those of rats maintained in sedentary condition without consuming comparably large amount of HF diet – the reward from running wheel activity appears to substitute for the reward derived from consuming a HF diet.

The reduced intake of palatable foods as a result of wheel running has been demonstrated in different exercise and feeding paradigms as well as in different strains of rats. Daily or alternative-day access to running suppresses intake of a 24% sucrose solution in

Sprague-Dawley rats (84). In Fisher 344 and Brown Norway hybrid rats, wheel running not only reduces intake/preference to a previously preferred HF diet (92), but also results in relative anorexia to both a familiar chow and a novel HF diet leading to severe weight loss (94). Furthermore, treadmill running reduces fat intake of weight cycling female rats (95). Taken together, previous findings and the data from Liang et al. indicate that, unlike the inconclusive results in human studies (87), exercise reduces intake and preference for highly palatable, energy dense food in rats. Data from Liang et al. further demonstrates that running suppresses weight gain even with HF diet available (91). The suppression of weight gain is not simply due to reduced intakes of chow and HF diet in WR rats compared to SED rats with similar diet availability because sedentary paired-fed rats that consumed equal amounts of chow and HF diets also gained significantly more weight than rats with running wheel access. Accordingly, the results of percent weight gain and energy intake among SED, WR, and paired-fed groups indicate that running sustains a relative negative energy balance even with ad lib access to chow and HF diets.

The Effects of Exercise on Obesity Models

Studies of individuals who have maintained significant weight loss for more than a year have demonstrated that dieters who achieve this long-term success are often those who engage in a regular and extensive exercise program (17). Whereas the energy expenditure aspects of such exercise surely contribute to the effects of weight maintenance, there is the suggestion that exercise may also contribute to energy balance by altering appetite and reducing food intake (18, 19). The effects of exercise on food intake and body weight have

been studied in a number of animal models of obesity, and the evidence suggests that such effects depend on the genetic cause of the obesity. For example, chronic exercise lowers the body weight gain and adiposity in diet-induced obese rats (20). In contrast, exercise has only minimal or no effect on the obesity of Zucker fatty or Koletsky corpulent rats, both congenitally lacking leptin receptors (21, 22, 23). Finally, exercise (presented at a specific age) can prevent the obesity of Otsuka Long-Evans Tokushima fatty (OLETF) rats lacking cholecystokinin (CCK)-A (or 1) receptors (24).

Shima and colleagues (25) originally demonstrated that providing OLETF rats with access to a running wheel helped to prevent their obesity by significantly decreasing their rate of body weight gain, normalizing their food intake, and having a preventive effect on the development of type II diabetes. Further study has demonstrated that the effects of exercise on the development of obesity in the OLETF rat are multiple and depend upon the age of the rats at the time that the opportunity for exercise is provided. In adult OLETF rats, access to a running wheel and the subsequent activity results in reductions in body weight down to the level of age matched control strain Long-Evans Tokushima Otsuka (LETO) rats. These changes in food intake and body weight are maintained as long as OLETF rats have access to running wheels (29). When access to the running wheel is discontinued, OLETF rats increase their food intake and their body weight increases and comes right back up to the level of aged matched OLETF rats that never had running wheel access. Thus, in the adult OLETF rats, exercise can ameliorate their obesity and normalize blood glucose and insulin levels (27). However, the effects of exercise in adult OLETF rats are temporary. Additionally, it has been shown that voluntary exercise can normalize altered feeding patterns and cause a variety of

changes in hypothalamic gene expression that may underlie the effects of exercise on food intake and body weight in OLETF rats. Together, these data suggest that voluntary exercise may activate hypothalamic pathways that overcome the deficits in CCK signaling that contribute to the hyperphagia and obesity in OLETF rats (29).

Access to a running wheel in young OLETF rats has different short- and long-term effects. When running wheel access is provided to OLETF rats at 6 weeks of age, the rate of body weight gain stops and weight remains stable until the OLETF rats reach the weight of aged matched LETO controls (28). They then continue to track the weight of LETO controls for as long as access to the running wheel is continued. In contrast, access to the running wheel and increased activity does not affect the rate of weight gain in LETO rats. They continue to gain weight at the same rate as sedentary LETO controls. When access to the running wheel is discontinued following 8 weeks of exercise in OLETF rats, some weight is regained but OLETF rats with past running wheel access never reach the weight of aged matched OLETF rats that never had running wheel access. Thus, there are lasting effects of exercise on the phenotype of OLETF rats (29).

Additionally, the changes in body weight in OLETF rats are not simply the result of increased energy expenditure. Although OLETF rats engage in more activity overall than LETO controls, running wheel activity also produces significant changes in food intake (28). Food intake of OLETF rats with running wheel access decreases sharply and remains at the same levels of LETO rats with running wheel access. When running wheels are relocked,

food intake again increases but does so only temporarily. When weight gain stabilizes, food intake is reduced and OLETF rats no longer demonstrate hyperphagia.

Another rodent model of human obesity is the diet-induced obesity (DIO) model. This rat model shares several common characteristics with human obesity and can be used to examine the effects of exercise on body weight gain and energy homeostasis (30). Similar to human obesity, rodent DIO is an inherited polygenic trait that is exacerbated by exposure to high fat, energy-dense diets and is accompanied by insulin and leptin resistance, hyperlipidemia, and hypertension (31). When outbred Sprague-Dawley rats are placed on a 31% high-energy (HE) diet, approximately half become hyperphagic and develop DIO, while the remainder are diet-resistant (DR) and do not become obese (32, 33).

Exercise begun early during the postweaning period in DIO rats can have permanent effects on lowering body weight gain, adiposity, and leptin levels for several weeks after exercise is terminated (36). In a study by Patterson et al. (37), results demonstrated that the obesity of DIO rats was completely prevented when the rats were given postweaning access to a running wheel, even when they were maintained on a high-fat/energy diet. Thus, 6 weeks of running wheel access prevented obesity for a subsequent 7 weeks in DIO rats on a high-fat/energy diet (38). Even 3 weeks of postweaning access to a running wheel was sufficient to significantly attenuate obesity development for a subsequent 10 weeks in DIO rats on a high-fat/energy diet (38). This ‘permanent’ lowering of the body weight set point is associated with long-term changes in hypothalamic neuropeptide expression and leptin signaling.

In summary, exercise can selectively lower the defended body weight and adiposity in DIO versus DR rats. Importantly, this reduction occurs in the absence of compensatory increases in food intake that would be expected based on their increase in energy expenditure. Furthermore, this effect is not dependent upon the amount of running wheel activity, but correlates highly with caloric intake. This suggests that rats monitor their energy balance not by their amount of activity, but by utilizing exercise-generated signals from the periphery that allow them to regulate caloric intake. Exercise and caloric restriction have an additive effect on lowering body weight gain and adiposity. Additionally, exercise produces a very different array of hypothalamic neuropeptide expression than caloric restriction (30), showing that exercise provides a unique mechanism for altering energy homeostasis. Unlike exercise in the adult rat, early-onset exercise during the postweaning period can produce long-lasting reductions in adiposity that persist even after exercise termination.

Activity-based Anorexia (ABA) Model

While exercise can have very positive effects on an individual's health, too much exercise can be detrimental. Anorexia nervosa (AN) is a severe psychiatric disorder that is characterized by continual self-starvation and critical, often life-threatening weight loss. The core diagnostic criteria of AN include a failure to maintain a minimal body weight, fear of gaining weight or becoming fat, and disturbances in perception of body shape or weight (1). Individuals with the restricting subtype of AN principally engage in dietary restriction, whereas individuals with the binge eating/purging subtype engage in dietary restriction followed by periods of binge eating and/or the use of inappropriate compensatory behaviors

(i.e., self-induced vomiting, laxative or diuretic abuse). Although excessive exercise is not considered a formal diagnostic criterion for AN, excessive physical activity appears to be a main aspect of the illness in some individuals (3, 4, 26), with up to 80% of individuals engaging in some form of extreme and/or demanding physical activity (3, 14, 26). The onset of the disorder typically occurs during early to mid-adolescence, with up to 90% of cases occurring in women (6, 7, 8, 12, 14). The course of AN is quite variable amongst patients, with some individuals recovering after just one episode while others endure a chronic course characterized by numerous episodes of recovery and relapse (9). There are few effective treatment options for anorexia nervosa (3, 9, 11), which unfortunately results in one of the highest mortality rates among any psychiatric disorders (9, 11).

Although dieting and a desire to be thin are extremely prevalent among adolescent girls, anorexia nervosa only affects approximately 0.5–1.0% of women in the general population (12). Thus, this low prevalence of anorexia nervosa in an environment with constant pressures to diet and be thin suggests that specific biological factors play a role in increasing the vulnerability of developing the disease in certain individuals. Throughout the course and development of AN there are a wide range of biological alterations that have been discovered, but it is unclear whether these alterations represent acute or chronic effects of starvation or whether they represent preexisting risk factors for the disorder. Consequently, an inherent confound in the clinical research of AN is the inability to identify at-risk individuals prior to the onset of disease. However, the use of animal models of anorexia nervosa enables the systematic study of biological factors involved in the development and maintenance of self-starvation in a controlled environmental setting. Obviously rodent

models of AN cannot represent all aspects of the clinical disorder, as rats can not have negative body image and diet in an attempt to lose weight. However, the use of an animal model of AN provides a systematic approach for studying specific hypotheses related to the neurobiology underlying self-induced food restriction, weight loss, and hyperactivity.

The most widely utilized animal model of anorexia nervosa is the activity-based anorexia (ABA) model, which was originally defined in 1953 by Hall and Hanford (13), and has since been used by many groups (7, 14). In this paradigm, rats on a restricted food access schedule (typically 1-2h per day), when given continuous, unlimited access to a running wheel, show increasingly greater levels of hyperactivity and decreased food intake during the 1-2h food access period, leading to a decline in body weight (58-61). Ultimately, if the protocol is not stopped after the rats lose approximately 25% of their original body weight (ABA criteria), which typically takes anywhere from 3-8 days, the rats will eventually die, thereby mimicking the phenomenon of severe self-starvation seen in the clinical population of AN (15). This model reproduces many of the core behavioral & physiological correlates of AN, including: restricted food intake in the presence of hunger, significant body weight loss, decreased body fat percentage, a decrease in body temperature, a drive for activity, and other physiological consequences (i.e. amenorrhea). Due to the fact that anorexia nervosa is most prevalent in teenage girls, the rats most often used in this paradigm are adolescent females. Adult female rats, as well as male rats, have also been used in this paradigm, but they typically do not display as severe a phenotype as the adolescent female rats (14, 218, 219).

There appear to be several interpretations as to the reasoning behind this paradoxical effect, one being a representation of foraging behavior whereby in times of short-term energy deprivation there is an increase in activity in order to find more food (62). An additional interpretation of this behavior attributes it to a reaction to peripheral energy balance signals such as leptin (63), in an attempt to thermoregulate in response to the change in ambient temperature (64, 55). Circadian influences should also be taken into account: caloric restriction usually entails giving the rodents food at a specific time each day. Because of a food-entrainable oscillator, the rodent will anticipate the time of food availability and consequently increase their activity in the time period before the food arrives (65); this sudden spike in activity in the 2-3 hours prior to scheduled food access is termed food-anticipatory activity (66).

Hyperactivity is observed in 40–80% of patients with anorexia nervosa (AN), depending on the way in which "hyperactivity" is operationally defined (3, 4, 14). This hyperactivity in response to limited food resources can be seen in various species, and an evolutionary hypothesis behind this behavior is based on the fact that these animals will display increased locomotor activity and travel large distances during periods of food scarcity in order to find new sources of food (17). As a result, the chance of survival of that species increases. This may be a partial biological explanation of why hyperactivity may be potentially rewarding. Indeed, reduced food intake and enhanced activity levels have been viewed as the principal symptomatology in AN, because only these behavioral measures consistently distinguish AN from other disorders (15-16). Increased running wheel activity upon exposure to the activity-based anorexia (ABA) model is displayed throughout the dark

phase, as well as in the light phase, in particular in the hours preceding food access. This activity in the light phase, which generally takes place 3-4 hours prior to scheduled feeding, is commonly referred to as food anticipatory activity (FAA) (14-16). Consequently, overall, if paired with restricted access to food, exercise can lead to unhealthy weight loss and can be viewed as a compulsive activity as opposed to a healthy way to improve body and mind.

Binge-eating Models Overview

In order to investigate the behavioral and neurological pathways involved in binge-eating disorder (BED), rodent models mimicking symptoms of the disorder have been developed. BED involves repeated, intermittent binges, which is defined as eating, in a discrete period of time (2hr), an amount of food that is definitely larger than most people would eat in a similar period of time under similar circumstances (1). According to the DSM-V, recurrent episodes of binge eating have to be present, occurring at least once a week for three months or more. Additionally, binge episodes have to be associated with at least three of the following: Eating more rapidly, Eating until uncomfortably full, Eating when not physically hungry, Eating alone because of embarrassment, and/or Feeling disgusted with oneself, depressed, or guilty after overeating. Binge eating in animals is characterized by behavior patterns similar to those seen in human binge eating (100).

To be classified as a binge, animals must consume large quantities of food in a brief, defined period of time, and this quantity should exceed that which would be consumed by control animals under similar circumstances (101). Binge eating behavior must be consistent and maintained over long periods of time (101). Crucial differences between animal and human BED include the fact that subjective feelings of distress or loss of control, which have been found in some (102, 103) but not all (104) studies linked prospectively to binge episodes in humans, are not easily assessed in animals (101). As such, animal models designed to study binge eating often exploit the precursory circumstances leading to binge

eating in humans (e.g., dieting, exposure to palatable foods and fluids, stress) to elicit binge-related behaviors (105, 106, 101, 107).

Some models use cycles of food restriction and refeeding (R/R) in order to induce bingeing, varying from 12h of food deprivation (96) to twice weekly episodes of 33% acute calorie restriction (97). Others use episodes of stress to augment intake. A variety of stressors, such as tail pinch, shock, and maternal separation, have been used to stimulate food intake in rodent models (98). Neonatal maternal separation, an animal model of stressful experiences in childhood, can permanently modify the hypothalamic-pituitary-adrenal (HPA) axis in the offspring (220), thus many researchers have used this model to mimic childhood stress in order to see its effects on the potential to develop an eating disorder (99). Stress-based models can be divided into the immediate, where the effect on intake is seen during or shortly after the stress, and the historic, where there is an extended period of time between the stress and intake assessment (101). Within these categories, the stress can be classified as acute, if it is applied only once, or chronic, if the animal has been repeatedly exposed to the stressor (101, 109). There are also various types of highly palatable foods (HPF) that are used in these bingeing studies, including vegetable shortening (108, 117), sucrose solutions (110, 111, 112), high fat diets (97, 114, 115), and cookies (98, 105, 115, 116). Four of the most prominently used models are: Limited Access, Sugar Addition, History of Dieting and Stress, and Food Restriction coupled with Limited Access. The various strengths and weaknesses of these models will be discussed in the following sections.

Limited Access

The limited access model was initially developed by Corwin and colleagues. This model involves repeated, intermittent access to a high fat food (hydrogenated vegetable shortening), as well as continuous ad libitum access to standard rodent chow, so as not to impose any form of food restriction on the animals (108, 117, 118). Specifically, animals are given shortening access every Monday, Wednesday, and Friday during the last 1-2h of the light cycle (depending on the experiment). With this model, animals maintained on intermittent access to Crisco display caloric over consumption within the access period relative to intakes of chow fed control animals as well as relative to intakes of animals that receive daily 2hr access or constant 24hr access to the shortening (117). Bingeing is operationally defined in this model when intake of the palatable food in the intermittent Mon, Weds, Fri group significantly exceeds that of the daily access group.

Particularly important to the development of this feeding pattern is the frequency by which animals have access to a highly palatable food (HPF). In particular, animals given daily limited access to vegetable shortening do not display the binge/compensate pattern of feeding; this feeding pattern only emerges when animals are given the Mon, Wed, Fri intermittent access to the shortening (108, 117). Furthermore, rats on the intermittent access schedule consume as much vegetable shortening during the brief access period as rats with continuous shortening access consume in 24-h (24 h/day–7 days/ week) (120, 118). Thus, there is something specific about the pattern of access, and while a HPF is necessary for bingeing to occur, it is not sufficient, and the neurological and behavioral results are unique.

Furthermore, this behavioral phenomenon has been reported in males and females, different strains, and across several age groups of rats (123, 117, 125, 124).

Rats with intermittent (Mon, Weds, Fri) brief access (1-2 h) to palatable food do not gain more weight, and do not accumulate significantly more body fat, than chow controls (123, 117). This is because of compensatory reductions in chow intake that occur. An overeat/undereat, or 'sawtooth' pattern of daily energy intake develops in the rats with intermittent access to palatable food because they overeat on days that palatable food is provided and undereat when palatable food is not provided (123, 117, 122, 129, 130). The net result is that total cumulative energy intake (chow + shortening) and body weight do not differ between intermittent access rats and chow controls (123, 117, 122, 129). The maintenance of energy intake and body weight at control levels is similar to human binge eating in which bingeing occurs, but body weight remains within the normal range because of compensatory behavior such as undereating at other times (1).

Numerous studies have shown that behavior directed toward consuming various foods is increased when the availability of these foods is decreased. For instance, non-food deprived rats drink more of a 1.5% w/v saccharin solution or 20% w/v alcohol when they are provided every other day as opposed to when they are provided on a continuous basis (118, 131).

Time-limited procedures typically do not promote a general overconsumption of intake, but rather promote brief sessions of excessive intake, i.e. bingeing. This is different

from procedures in which extended access promotes cumulative overconsumption, such as for a high fat diet, in which extended access induces sustained hyperphagia and obesity (132). To sum up, consumption of a variety of items can be altered by maintaining a constant session duration while altering session frequency (3 times a day, daily, etc.), or by altering session duration and keeping session frequency constant (118).

Limited access to a highly palatable food in these binge-eating models has relevance to the "forbidden foods" hypothesis (133), suggesting that foods upon which people binge are those to which they have limited their own access. "Forbidden foods" are typically high in fat and/or sugar, and individuals who are attempting to lose weight frequently restrict their access to these types of foods, but consume them in large quantities during a binge. Thus, due to the fact that they severely restrict their own consumption of these specific foods, it makes them all the more desirable.

Results from rat experiments by Corwin and colleagues suggest that occasionally eating small quantities of highly preferred fatty foods may protect against subsequent bingeing to some degree (118). Several other interesting findings have been reported as well: 1) Limiting the quantity of shortening consumed during a 1-h period of availability for five weeks produced robust and long-lasting decreases in subsequent binge size when quantity was no longer restricted under intermittent availability conditions. 2) Five weeks of unrestricted access to shortening also produced robust and long-lasting decreases in subsequent binge size. 3) Five weeks of no access to shortening, on the other hand, produced rapid and long-lasting increases in subsequent binge size. This study demonstrated that

intermittent access is necessary and sufficient to promote bingeing on fat, but the size of the binge can be altered by the fat-access history (118).

Activation of brain reward signaling has been implicated in promoting and sustaining binge-like behavior in animals. In the limited access model, roles for activation of Dopamine-1 (D1) and Dopamine-2 (D2) receptors have been tested (124). Administration of the D1-like antagonist SCH23390 reduced intake of fat in binge and control rats, but these results were often also accompanied by reductions in chow intake (119). Therefore, the effects of D1 blockade may have been due to generalized suppression of feeding behavior. However, administration of the D2 antagonist raclopride had specific effects on consumption of fatty palatable foods. Specifically, intake of a fatty palatable food was generally reduced by raclopride at relatively high doses in rats with daily limited access but was either unaffected or increased by raclopride at lower doses in rats with intermittent access (119). These results implicate D2 receptors in the consumption of fatty food, but also indicate differential D2 signaling in binge-eating rats and controls. Since lower doses stimulated intake in the binge (sporadic) rats and higher doses reduced intake in the controls, these results have been interpreted to suggest differential pre- and post-synaptic D2 signaling under binge and control conditions. These findings are consistent with reports in humans and in rats implicating altered DA signaling in the consumption of fatty foods (134) and in binge eating (135, 113).

Overall, the limited access protocol provides a means of establishing elevated intakes of a highly palatable, binge-type food in discrete periods of time in non-food deprived rats

for extended amounts of time (101). It includes a voluntary compensatory component and the dopamine aspect of the brain reward pathway appears to play a role in this bingeing behavior. The phenomenon is not only robust, but also quite reliable, as Corwin et al. have demonstrated it in different strains and ages of rats, in males and females, and in mice (117, 123 125). The Corwin limited access protocol seems to be a strong isomorphic model, with good construct validity.

Sugar Addiction

Another model of binge eating that has been utilized, particularly by Avena, Hoebel and colleagues, involves sugar instead of fat as the source of the highly palatable binge-type food. Using this method, binge-eating behavior is observed in rats after just a few days of discontinuous access to a sugar solution (e.g., 25% glucose or 10% sucrose) and chow (112). In this paradigm, rats have daily access to sugar and chow for 12 h followed by 12 h of food restriction, and this schedule is maintained for approximately 1 month (136). In this model, binge-eating behavior is defined as an increase in consumption of the sugar solution during the first hour of access; it has been demonstrated that animals can consume roughly 20% of their total daily sugar intake in the first hour of access. Additionally, sugar-bingeing rats gradually increase their total daily intake of sugar, eventually drinking as much in the 12 h access period as ad libitum-fed rats do in 24 h (~70 mL/day). In order to induce bingeing to the greatest extent and ensure that the rats will be hungry when the food is made available, a 4-h delay between the onset of the dark cycle and the onset of food access is imposed, as rats typically begin eating at the onset of the dark cycle (136).

Body weight is not significantly different between sugar-bingeing rats and rats with ad libitum access to chow or sugar. These rats are able to regulate their caloric intake and compensate for the excess calories acquired from sugar by eating less chow (137). This type of self-imposed bingeing/restricting behavior is similar to that of some patients with non-purging type bulimia (1), particularly those who binge eat, but are still able to maintain a healthy body weight.

Food is a natural reward that activates neurochemical pathways in the brain that evolved in order to reinforce this behavior. Other reinforcers, such as various drugs of abuse, hijack these brain circuits in order to exercise their reinforcing effects (138, 139, 53). Taken together, this similar brain circuitry, in addition to self reports from individuals describing feelings of compulsion to eat fatty and/or sweet foods, which can be interpreted as being somewhat similar to an addict's compulsion to smoke cigarettes or drink alcohol, has stimulated the study of what is being termed as "food addiction" (53). The sugar binge eating model described here is a tool that was established to be used to study food addiction in the laboratory (136).

Rats maintained on this sugar binge protocol demonstrate neurochemical alterations in the underlying reward system, including an increase in mu-opioid and D1 receptor binding in the nucleus accumbens (NAc), as well as increased D3 receptor mRNA expression in the NAc (140, 141). The NAc is one of the brain regions involved in motivation and reward for both eating and drug abuse (142, 143). Studies using in vivo microdialysis have shown that sugar-bingeing rats release dopamine (DA) in the NAc on days 1, 2, and 21 of bingeing on

sugar, while non-bingeing rats show a blunted DA response that is similar to the effect seen with a palatable food that is no longer novel (144). These neurochemical changes are similar, although smaller in magnitude, to what is observed when rats repeatedly administer drugs of abuse (136).

After binge-eating sugar for approximately 1 month, rats show signs of opiate dependence. The opioid antagonist naloxone can be used to precipitate withdrawal signs, such as teeth-chattering and tremors in the sugar bingeing rats (145, 137). Signs of withdrawal can also emerge spontaneously by fasting the rats for 24–36 h (137). In both of these cases, the withdrawal behavior is coupled with an increase in the release of accumbens acetylcholine (ACh) and a decrease in the release of DA within the NAc (145, 137). This neurochemical imbalance in DA and ACh has also been seen during withdrawal from certain drugs of abuse, including alcohol, morphine, and nicotine (146, 147). Additionally, following abstinence from sucrose, rats will exhibit a larger binge than they typically demonstrate, indicating a "deprivation effect" and suggestive of craving (110).

Sweet-fat addiction

Another, similar model of binge eating, developed by the same researchers as the sugar addiction model, is one in which limited daily access to sweet-fat chow in non-deprived animals leads to bingeing behavior (130, 148). Unlike Corwin's model which uses pure fat, this protocol uses a sweet-fat diet, with the goal of capitalizing on the documented effects that sugar bingeing can have on behavior and the brain, and combining them with the

effect that fat is expected to have on body weight. Rats that have daily 2 h access to sweet-fat chow (45% fat, 20% protein, 35% carbohydrate, 4.7 kcal/g) binge on it, even though they have ad libitum access to standard rodent chow for the other 22 h of the day. By week 3 of scheduled access, rats typically consume roughly 58% of their daily calories during the 2 h sweet-fat chow access period (130).

Although bingeing on a sugar solution does not lead to obesity, binge eating a sweet-fat diet does result in significant changes in body weight (130). These animals demonstrate daily self-imposed restriction of standard chow intake, resulting in fluctuations in daily body weight illustrated by weight loss between binges. A sawtooth pattern emerges for the 2 h Daily Sweet-fat group in which they decrease in weight pre-binge and increase in weight post-binge each day. However, despite these alterations in body weight, rats with daily 2 h access to a sweet-fat diet weigh significantly more than control groups that either have continuous chow or sweet-fat chow available ad libitum. Moreover, this binge eating model reflects another component of human binge eating, which is eating in the absence of hunger, which contributes to the validity of the model because food restriction is not driving the increased food intake. The combination of sugar and fat comprises a large proportion of the snacks and desserts that patients with eating disorders often overconsume, frequently contributing to body weight gain (149, 150 , 151, 152).

History of Dieting and Stress

This model of binge eating, developed by Boggiano and colleagues, involves periods of food restriction combined with stress prior to access to a palatable food. This model takes into account the considerable evidence of a history of food restriction (1, 153, 154) and environmental stress in the precipitation and maintenance of binge eating (1, 155, 156, 157, 77). In the nonclinical population, food restriction is consistently the strongest predictor of overeating in response to stress (158, 159, 160). These observations suggest that certain aspects of binge eating may be caused by a particular interaction between dieting and stress.

Several studies have examined the onset of dieting in relation to binge eating among adult samples with BED, and have found that roughly 35-55% of adult clinical and nonclinical samples reported that the onset of binge eating occurred after experience with dieting and restrained eating (166). Restrained eating refers to the voluntary cognitive effort to restrict food intake to control body weight (167). Many studies have shown that cognitive restraint is a consistent predictor of overeating under stress, in particular, with highly restrained eaters increasing and free eaters decreasing their food intake under stressful conditions. Additionally, in clinical practice, obese patients report stress as a primary trigger for binge eating (168).

One possible contributing mechanism underlying stress-induced eating involves the activity of the HPA axis during stress, particularly the release of glucocorticoids (GCs) from the adrenal cortex. Sapolsky has proposed that food intake is inhibited by corticotrophin

releasing hormone (CRH) but promoted by GC production (169). Furthermore, the release of GC during stress has been associated with increased snack intake. In one study, fifty pre-menopausal women completed a laboratory stressor, provided saliva samples to assess cortisol levels and then completed daily hassles and snack intake diaries over the next fourteen days. There was a significant association between daily hassles and snack intake within the overall sample, suggesting that during times of increased stress these women consume a greater amount of high calorie snacks (169). Additionally, snack intake was significantly associated with dietary restraint, emotional eating, disinhibition, and external eating in the overall sample. Furthermore, the results indicate that eating response to stress differed between high and low cortisol reactor groups, such that high cortisol reactors consumed a greater amount of food, especially high fat, sweet foods (221). Moreover, it has been demonstrated that individuals with bulimia and BED tend to show either greater basal cortisol or greater cortisol reactivity, as reviewed by Gluck (168, 167, 170). In a small but well controlled laboratory stress study, BED compared to control women tended to have greater cortisol reactivity and desire to binge after a cold pressor lab stressor (168, 167).

Another possible mechanism for the effect of stress on food intake hinges on the idea that stress as well as palatable food can stimulate endogenous opioid release. In turn, opioid release appears to be part of an organisms' powerful defense mechanism protecting against the detrimental effects of stress by decreasing activity of the HPA axis and thus attenuating the stress response (167). Repeated stimulation of the reward pathways through either stress induced HPA stimulation, intake of highly palatable food, or both may lead to neurobiological adaptations that promote the compulsive nature of overeating.

Boggiano and colleagues developed their particular binge model to more accurately represent this specific aspect of the clinical binge eating population. Thus, their model incorporates environmental stress but demonstrates hyperphagia in animals that are not in energy deficit because eating without hunger and normal body weight are typical of people who binge eat (1, 162, 163). With this model, rats are subjected to brief cycles of caloric restriction followed by ad lib refeeding (at least three cycles) prior to being exposed to an acute episode of stress via foot shock. In keeping with the somewhat higher prevalence of binge eating disorder (BED) in young adult females (1), young female rats were used. To test for a significant interaction between dieting and stress, intensities and durations of both food restriction and stress were used that alone did not affect food intake relative to control rats. To that end, there was no cumulative effect of a history of restriction on food-intake in rats restricted multiple times but never shocked, nor of repeated experience with foot shock without restriction. Hence, the hyperphagia appears to involve a unique interaction that does not occur with either stress alone or restriction-refeeding cycles alone.

In conclusion, this protocol provides additional evidence that a history of caloric restriction and stress, despite ad lib chow intake and normal body weight, interact to promote abnormal increases in food intake that are characteristic of binge eating. Additionally, it was shown that stress after restriction-induced overeating produces a clear preference for highly palatable food over chow. This pattern of selection is consistent with feeding motivated by reward as opposed to metabolic need or energy-regulation (164, 165). If hunger played a key role in the stress-induced overeating, restricted rats would overeat chow when only chow is available but they do not. These results parallel the role of highly palatable food and hunger

as binge-precipitating factors in humans and strengthen the validity of this animal model to further our understanding of binge-eating disorders.

Food Restriction coupled with Limited Access

Another novel rodent model of binge eating, developed by Bello and colleagues, incorporates intermittent acute food restriction (33% of the previous day's chow intake) followed by brief periods of access (2 h) to a highly palatable sugar-fat mixture (97). These repeated episodes of acute caloric restriction are in contrast to what have been used in other binge-feeding paradigms, which use extended periods of caloric restriction [e.g., 66% calorie restriction for 5 days (171) or 12 h of daily restriction for 8–30 days (145, 140)]. The sweet-fat diet used in this model consists of 90% vegetable shortening (Crisco) and 10% sucrose, which is characteristic of bulimic binges because it is high in fat and sugar (152). In addition, the binge-access period occurs during the period of active eating in the rat (i.e., the dark portion of the lighting cycle) during which the greatest amounts of food are typically consumed, which resembles intakes in BED and BN patients, who generally binge more during afternoon and evening meal times (152, 172). Furthermore, the binge-access rats in this model are exposed to intermittent restriction days alternating with two binges per week, thereby modeling the intermittent nature of binge behavior demonstrated in BED and BN patients.

In this protocol, rats are divided into three experimental groups: continuous-access, binge-access, and chow-restricted groups. The continuous-access group has unlimited

optional access to standard chow and sweetened fat throughout the experiment (97). The chow-restricted and binge-access groups are restricted at the beginning of the dark cycle to 33% of the previous day's chow caloric intake on days 2 and 5 of each week (173). On restriction days, animals consumed the entire restricted amount of chow within the first 4 h. Hence, on the restriction days, animals experienced 20 h of food deprivation before refeeding. On subsequent days (days 3 and 6) 2 h into the dark cycle, the binge-access groups were given access to standard chow and the sweetened fat, whereas the chow-restricted groups were refed chow alone. The binge-access group had access to sweetened fat for only the first 2 h of each refeeding period. In this fashion, the binge-access group was exposed to a repeated cycle that consisted of a no-restriction day, a day of calorie restriction (days 2 and 5), and a day of scheduled refeeding starting with 2 h of access to an optional palatable food.

Bello and colleagues demonstrated that the binge-access group consumed approximately 170% more calories of the sweetened fat plus chow during the 2 h refeeding period than during the subsequent 20 h chow-only intake on binge days and approximately 75% of the calories consumed during the 24 h chow only non-restriction days (97). Because the eating patterns occur in repeated episodes and the calorie amount eaten in a short amount of time (2 h) approximates the rat's daily (24 h) caloric intake, the amount consumed in the measured time period is binge-like. Data from these experiments demonstrates that repeated acute calorie restriction before access to a highly palatable food results in an exaggerated binge-like behavioral response in adult male Sprague-Dawley rats.

Rather than examining factors that initiate or lead to an onset of binge eating, Bello and colleagues used a restriction/binge model of BN to determine the physiological consequence of this pattern of eating behavior that may facilitate the maintenance of the behavior. Accompanying the alteration in palatable food preference are hormonal and neuronal changes that promote the maintenance of the behavior. In addition to their well-described action in the striatum, opioids have been demonstrated to be involved in feeding-related actions in other brain regions. This raises the possibility of opioid dysfunction at multiple brain sites as a consequence of dietary conditions. Studies by Bello and colleagues have investigated the involvement of the caudal brainstem opioid system in dietary-induced binge eating (114). In particular, the nucleus tractus solitarius (NTS) is a critical hindbrain structure involved in the integration and control of food intake. Mu-opioid receptors are distributed throughout the NTS and vagus nerve. Several studies have suggested that NTS opioid signaling engage forebrain regions to modulate food intake. Although circulating peripheral hormones (e.g., leptin, ghrelin, glucagon-like peptide-1) modulate caudal brainstem feeding responses and body weight, sensory information (i.e., mechanical, chemosensory) involved in the negative feedback control of meals and gastrointestinal input is conveyed predominantly via vagal afferents. Increases in gastric capacity, alterations in meal-related hormones, and reductions in subjective satiety ratings have been reported in BN and BED. Such findings suggest that gastrointestinal integrative functions are likely altered in BN and BED to accommodate the overeating and reduce satiety experienced by these patients.

After six weeks, animals on this binge access schedule had greater meal-induced neuronal activation, as measured by immunoreactive c-Fos, in the medial region of the NTS (114). Additionally, binge access animals had reduced mu-opioid receptor expression in the NTS compared with scheduled access and naive controls. A similar reduction in mu-opioid receptor mRNA was also demonstrated in the continuous access group. Not only did the continuous access group have reductions compared with scheduled access and naive groups, but reductions were also demonstrated in comparison with the chow restricted group. In the nodose ganglion group differences were also apparent for mu-opioid mRNA levels. The chow restricted group demonstrated increased mu-opioid receptor mRNA compared with binge access, continuous access, and naive controls. Taken together with the previous findings of increased neuronal activation in the NTS following a meal of the Binge Access rats (97), the downregulation of mu-opioid receptor mRNA could likely have also resulted from increased activity as a consequence of the bingeing schedule.

Summary

Upon weighing all the pros and cons of each binge-eating model, I chose to use a modified version of Crowin's limited access model. I believe this model more accurately reflects human binge-eating, partially because no form of food restriction is placed on the rats and thus there is no potential for a food restriction-induced artificial increase in food intake that is mistakenly classified as binge-eating behavior. I also did not want to use a model that incorporates food restriction because the experiments that I performed examined the effects of running on binge eating, and it has been shown that a combination of food

restriction and access to a running wheel can potentially lead to anorexia (i.e., the activity-based anorexia model) and disordered eating. Additionally, just as in human binge-eating, the access to highly palatable food is intermittent, occurring approximately every couple days as opposed to continuous access. The palatable food is also only available for a short period of time (1-2h), which also accurately reflects the duration of time that humans typically spend bingeing. Importantly, rats in this model consume large amounts of highly palatable food in short periods of time (1-2h), above and beyond controls that have either daily 1h access or 24h continuous access to vegetable shortening.

Overall, animal models of binge eating have focused on identifying factors that contribute to the development and maintenance of binge behavior. Roles for the palatability of the binge food, intermittent access, dieting or periods of food restriction, and stress have been identified. Once binge behavior is established, its consequences on brain reward systems can be studied and such changes may provide insights into mechanisms that sustain this disordered eating behavior and make it difficult to treat (67, 72, 85).

Chapter 2. Experimental Background

Binge eating disorder (BED) is the most common eating disorder, with a lifetime prevalence of 3.5% among women and 2% among men, with an average time of onset in early adulthood. BED has been formally recognized as a specified eating disorder in DSM-5 (1). BED involves repeated, intermittent binges, which is defined as eating, in a discrete period of time (2hr), an amount of food that is definitely larger than most people would eat in a similar period of time under similar circumstances. This behavior is also accompanied by a feeling of loss of control, whereby individuals feel unable to stop themselves from eating. Typically, high fat, sugar, and/or carbohydrate foods are consumed during binge episodes, but these foods are normally avoided under non-binge circumstances (2, 133). Additionally debilitating is the fact that this disorder is associated with a variety of mental and physical health problems (2, 133).

BED is considered one of the more difficult psychiatric conditions to treat, partially due to the additional co-morbid physical and psychiatric ailments. Some therapies, such as Cognitive Behavioral Therapy (CBT), are effective for some but not all binge-eating patients, and only recently has one medication (Vyvance) been approved for specific treatment of this disorder (35). Due to the fact that weight issues, body dissatisfaction, and physical inactivity can be central issues in BED, exercise therapy may be a useful treatment. In fact, two randomized control trials in humans indicate that BED patients who follow an aerobic exercise program report fewer binges per week or even completely abstain from bingeing entirely (88, 183, 184).One study by Pendleton et al. demonstrated that a combination of

aerobic exercise and CBT led to fewer binges per week than CBT alone, suggesting that even mild exercise could have an added therapeutic benefit above and beyond current treatment standards (88, 183, 184).

Unfortunately, there has been limited progress in the development of treatment strategies due, in part, to the fact that the neurological and physiological consequences of repeated bingeing are not clearly understood and cannot readily be studied in humans. Thus, well developed animal models may aid in our understanding of binge eating. In order to investigate behavioral and neurological pathways affected by BED, a rodent model mimicking symptoms of the disorder can be used. The model used here is very similar to the Corwin limited access model, involving repeated, intermittent access to a high fat food (hydrogenated vegetable shortening), as well as continuous access to standard chow, so as not to impose any form of food restriction on the animals. Specifically, animals are given shortening access every Monday, Wednesday, and Friday during the last 1hr of the light cycle, immediately preceding the onset of the dark cycle. We use male, early adult aged rats in our model, which mimics the patient population fairly well because unlike other eating disorders, BED is common in both sexes. Using this animal model we are able to mimic binge eating behavior. However, we are unable to measure the animals' potential feelings of loss of control.

One important point to make is that the behavioral and neuronal consequences of bingeing on a highly palatable food (HPF), even when at normal body weight, are different from those that result from simply consuming a HPF in a non-binge manner. In Corwin and

colleagues' binge eating model, animals maintained on an ad libitum standard rodent chow diet and given intermittent (approximately every 3 days) 2hr access to vegetable shortening (Crisco) display caloric over consumption relative to chow fed control animals as well as animals that receive daily 2hr access or constant 24hr access to the shortening (126). Particularly important to the development of this feeding pattern is the frequency in which animals have access to the HPF. In particular, animals given daily limited access to vegetable shortening do not display the binge/compensate pattern of feeding; this feeding pattern only emerges when animals are given intermittent access to the shortening (126,101). In this way, animals given limited access to the vegetable shortening not only consume more calories than chow fed control animals during the binge period, but also more than animals receiving daily 2hr access to vegetable shortening. Thus, there is something specific about the pattern of consumption, and while a HPF is necessary for bingeing to occur, it is not sufficient, and the neurological and behavioral results are quite unique.

Various other rodent models of binge eating have been used, which differ in many aspects from Corwin's model. Some models use degrees of food restriction and refeeding (R/R) cycles in order to induce bingeing, varying from 12hr of food deprivation (96) to twice weekly episodes of 33% acute calorie restriction (97). Others use episodes of stress to augment intake. A variety of stressors, such as tail pinch, shock, and maternal separation, has been used to stimulate food intake in rodent models (98). Stress-based models can be divided into the immediate, where the effect on intake is seen during or shortly after the stress, and the historic, where there is an extended period of time between the stress and intake assessment (101). Within these categories, the stress can be classified as acute, if it is

applied only once, or chronic, if the animal has been repeatedly exposed to the stressor (101, 109). There are also various different types of highly palatable foods (HPF) that are used in these bingeing studies, including vegetable shortening (108, 117), sucrose solutions (110, 111, 112), high fat diets (97, 114, 115), and cookies (98, 105, 115, 116).

Chapter 3: Experiment 1- General Effects of Running Wheel Activity on Binge Eating

Abstract:

Binge eating disorder (BED) is an eating disorder involving repeated, intermittent over consumption of food in brief periods of time, usually with no compensatory behaviors. There are few successful treatments and the underlying neural mechanisms remain unclear. In the current study, we hypothesized that voluntary wheel running activity could reduce binge eating behavior in a rat model. Rats were given intermittent (3 times/wk) limited (1hr) access to a high-fat food (Crisco), in addition to continuously available chow. Crisco was available every Mon, Wed, and Fri for 1h before dark onset. Rats were divided into 2 groups: those with (RW) and those without running wheel access (SED). Based on week 1 Crisco intake, both groups were divided into Binge Eating Prone (BEP) and Resistant (BER) animals. Binge intake escalated in both the SED and RW groups. Within both SED and RW groups, the BEP rats ate significantly more Crisco than the BER rats. There was no difference in Crisco intakes of the BEP rats between the SED and RW groups, but the RW BER rats ate significantly less Crisco than the SED-BER rats. There was a significant increase in PFC Oprm1 mRNA in the SED BEP group relative to SED-BER, as well as decreased NAc Oprm1 mRNA and a trend toward increased TH mRNA expression in the VTA of the RW-BER rats. These results were not consistent with our overall hypothesis in that RW access did not decrease Crisco intake in the BEP rats. Overall, access to a RW and the resulting activity reduced binge behavior in the RW-BER animals as well as modulated the effects of bingeing on brain reward systems.

Introduction:

In rodents, acute running wheel activity decreases food intake and body weight for several days (75). When rodents are provided with running wheel access for a longer period of time, food intake is increased to compensate for the increased energy expenditure but the body weight remains lower than that of sedentary controls (90). One potential mechanism that could contribute to body weight control with exercise is through altering dietary preferences. Rats introduced simultaneously to a sucrose solution and running wheel engage in running wheel activity but avoid consuming the sweet solution (84). When a solid high fat diet (HFD) is provided, wheel running can significantly reduce HFD intake and consequently decrease the preference for a previously preferred HFD in a two-diet (standard rat chow vs. HFD) choice feeding schedule (91, 92). Additionally, alternative-day access to a running wheel has been shown to suppress intake of a 24% sucrose solution in rodents (84).

To date, no studies have been published examining the effects of voluntary exercise on binge eating behavior in rats. It has been shown that running wheel activity (RWA) can decrease consumption of a daily high fat diet, but the effects of RWA on sporadic, intermittent consumption of a HFD (e.g. binge eating) are still relatively unknown. Thus, this study aims to examine some of the behavioral and neuronal consequences of activity on binge eating.

In the current experiment, we investigate how voluntary running wheel access affects binge behavior within a limited access rat model of binge eating. We hypothesize that wheel running would reduce binge eating behavior in rats, specifically by reducing Crisco

consumption. In order to evaluate the mechanisms underlying vulnerability to binge eat and the effects of running wheel access, we measured the expression of several genes involved in food reward and reinforcement, including: tyrosine hydroxylase (TH), the mu-opioid receptor (Oprm1), and the dopamine-2 receptor (D2R). We analyzed these genes within various brain reward regions, including: the prefrontal cortex (PFC), the nucleus accumbens (NAc), and the ventral tegmental area (VTA). These genes were chosen because it has been shown in other rodent studies that binge-eating can activate the reward system, which often involves the release of dopamine and endogenous opioids (113, 114, 213, 101, 124). Additionally, imaging studies show that individuals with BED might have impairments in dopaminergic and opioid pathways that regulate neuronal systems associated with reward sensitivity, conditioning, and control (5).

Methods:

Animals

A total of forty-two adult male Sprague Dawley rats from Harlan were obtained when they were between fifty to sixty days old and weighed 220-240g. They were individually housed in plastic tub cages in a temperature- and humidity-controlled room under a 12:12-h light-dark schedule (lights off from 12PM to 12AM). All rats had ad libitum access to water and standard laboratory rodent chow (Harlan 2018, Fredrick, MD; 3.1 kcal/g: fixed formula diet of 18.6% protein, 44.2% carbohydrate, and 6.2% fat). All animal procedures were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine.

Experimental design

All rats had unlimited access to the standard chow diet and water throughout the study. At the end of the 1-week adaptation period, seven days prior to week 1 Crisco access, all rats were given overnight access to a jar of hydrogenated vegetable shortening (Crisco® brand All-Vegetable Shortening, J.M. Smucker Company), clipped to the front of the cage. Rats were then assigned to one of two groups: those with (RW) (n=21) and those without continuous running wheel access (SED) (n=21), with no significant mean differences in baseline overnight Crisco intake, chow intake, or body weight. Additionally, five days prior to week 1 Crisco access, the RW rats began habituating to their running wheels and continued to have unlimited running wheel access throughout the four week experiment. Subsequently, rats were given intermittent (3 times/wk) limited (1hr) access to a high-fat food (Crisco®), in addition to continuously available chow and water. Crisco was available

every Monday, Wednesday, and Friday during the last 1hr of the light cycle, immediately preceding the onset of the dark cycle.

One- and 24-h intakes of shortening and standard chow, respectively, were measured every day throughout the study by weighing the shortening and standard diet and subtracting the current food weight from the most recent previous food weight. Body weights were measured daily. Running wheel activity (RWA) was also recorded daily using a cage registration program (VitalView, Mini Mitter, Bend, OR). RWA was assessed during various time periods: the light cycle, dark cycle, total daily RWA, during the 1h Crisco access time period, and during the food-anticipatory period. Food anticipatory activity (FAA) is an increase in locomotor activity preceding the presentation of food (specifically Crisco in this case) that develops when food is available during a restricted and predictable time of the day (179). In this study, FAA was classified as the RWA during the three hour time period prior to the Crisco access. On the Monday of week 5, all rats were decapitated 1h prior to regularly scheduled Crisco access. The brains were collected and stored at -80°C. Brain punches were taken of the Prefrontal Cortex (PFC), Nucleus Accumbens (NAc), and Ventral Tegmental Area (VTA), and RT-PCR was performed to determine mRNA expression.

Binge groups

Based on Week 1 (W1) Crisco intake, both groups were divided into Binge Eating Prone (BEP), Neutral (BEN), and Resistant (BER) animals. In each group, the rats that consistently consumed more than average Crisco (greater than 2 standard errors above the mean BEN Crisco intake) were labeled BEP and those that consistently consumed less than average Crisco (greater than 2 standard errors below the mean BEN Crisco intake) were

labeled BER. Those that consistently ate close to the group average of Crisco (less than 2 standard errors from the mean BEN Crisco intake) were labeled BEN, and were not used in the analysis. Rats were ranked from low to high on their 1h Crisco intake on each of the first three binge days (Mon., Weds., and Fri. of week 1). The rankings were combined across the three days and based on these rankings, rats were separated into sedentary BER (SED BER; n=7), sedentary BEP (SED BEP; n=8), RW BER (n=11), and RW BEP (n=6).

Within the SED group, there were eight rats that were categorized as BEP, with an average weekly (W1-4) Crisco intake of 30.6 kcal +/- 1.94 (SE), seven rats categorized as BER, with an average weekly (W1-4) Crisco intake of 16.42 kcal +/- 0.84, and six rats categorized as BEN, with an average weekly (W1-4) Crisco intake of 24.6 kcal +/- 1.4. Within the RW group, there were six rats that were categorized as BEP, with an average weekly (W1-4) Crisco intake of 38.5 kcal +/- 2.99 (SE), eleven rats categorized as BER, with an average weekly (W1-4) Crisco intake of 6.42 kcal +/- 0.76, and four rats categorized as BEN, with an average weekly (W1-4) Crisco intake of 17.22 kcal +/- 2.4.
These binge prone and resistant groups remained consistent throughout the four week study such that the rats categorized as BEP (based on the week 1 rankings) consistently consumed more Crisco than the rest of the rats throughout the study, and the rats categorized as BER (based on the week 1 rankings) consistently consumed less Crisco than the rest of the rats throughout the study.

mRNA expression

The Prefrontal Cortex (PFC), Nucleus Accumbens (NAc), and Ventral Tegmental Area (VTA) were isolated from 500 μm thick frozen coronal sections using a Harris uni-core tissue puncher (ID 0.75 mm) (Ted Pella, Inc., Redding, CA, USA) based on the coordinates for adult rat brains (180). The RNeasy Lipid Tissue Mini Kit with the Qiazol reagent (Qiagen, Valencia, CA) was used to isolate total RNA from the tissue punches. 200ng of RNA was used to generate cDNA for subsequent quantitative real-time PCR with a QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA). All reactions were carried out in triplicate using 1X Taqman master mix (Applied Biosystems, Foster City, CA), cDNA template, and 1X Taqman probes for each gene (Oprm1, D2R, TH, and Actb) (Life technologies, Grand Island, NY) in a total volume of 20μL. Real-time reactions were performed with standard PCR conditions (50°C for 2 min; 95°C for 10 min; and 95°C for 15 sec and 60°C for 1 min for 40 cycles) on an Applied Biosystems 7900HT Fast Real-Time PCR System. Each set of triplicates was checked to ensure that the threshold cycle (Ct) values were all within 1 Ct of each other. Negative RT samples were used to control for possible contamination of cDNA. To determine relative expression values, the -ΔΔCt method (Applied Biosystems, Foster City, CA) was used, where triplicate Ct values for each tissue sample were averaged and subtracted from those derived from the housekeeping gene Beta-actin (Actb). The average Ct difference for the control group (sedentary BER rats) was subtracted from those of the test samples, and the resulting -ΔΔCt values were raised to a power of two to determine normalized relative expression.

Statistical Methods

Data are presented as averages with standard error of the mean. Statistical analyses were performed using Statistica 7 (Systat Software Inc, Tulsa, OK) and SigmaPlot 11.0 (Systat Software Inc) software. Group differences in body weight, chow intake, Crisco intake, total intake, Crisco/Total intake, and running wheel activity were assessed with three-way ANOVA with running wheel exposure (GROUP) and binge eating status (BINGE) as the between-subjects factors, and time as the within-subjects factor. Subsequent analyses included: entire RW group vs entire SED group; all the BER animals from both the RW and SED group vs all the BEP animals from both the RW and SED group; RW (BER v BEP) v SED (BER v BEP). Main and interaction effects were identified. Differences at specific time points and between specific experimental groups were assessed by Tukey's post-hoc tests. Group differences in mRNA expression were also assessed by ANOVA followed by Tukey's post-hoc tests. For all statistical analysis a confidence interval of 95% was used. Finally, we evaluated potential relationships between: the expression of various genes and Crisco intake, running wheel activity (RWA) and Crisco intake, and RWA and chow intake using Statistica.

Results:

Crisco intake, Body weight, Chow intake, Total intake, Crisco/Total intake, and running wheel activity

There was no overall significant effect of RW access on Crisco intake ($F(1, 112) = 0.505$ $p=0.479$) (Fig 1). However, there was a significant time*group interaction ($F(3, 112) = 5.468$ $p=0.002$) whereby initially the RW group had lower Crisco intake in week one but gradually increased their Crisco intake so that they eventually ate an amount similar to that consumed by the Sedentary group (Fig 2). There was an overall significant binge effect ($F(1, 112) = 237.95$ $p<0.001$), whereby the BEP rats ate more Crisco overall compared to the BER rats. There was also a group*binge interaction ($F(1, 112) = 35.55$ $p<0.001$), and a significant group*binge*time three-way interaction ($F(3, 112) = 3.718$ $p=0.014$).

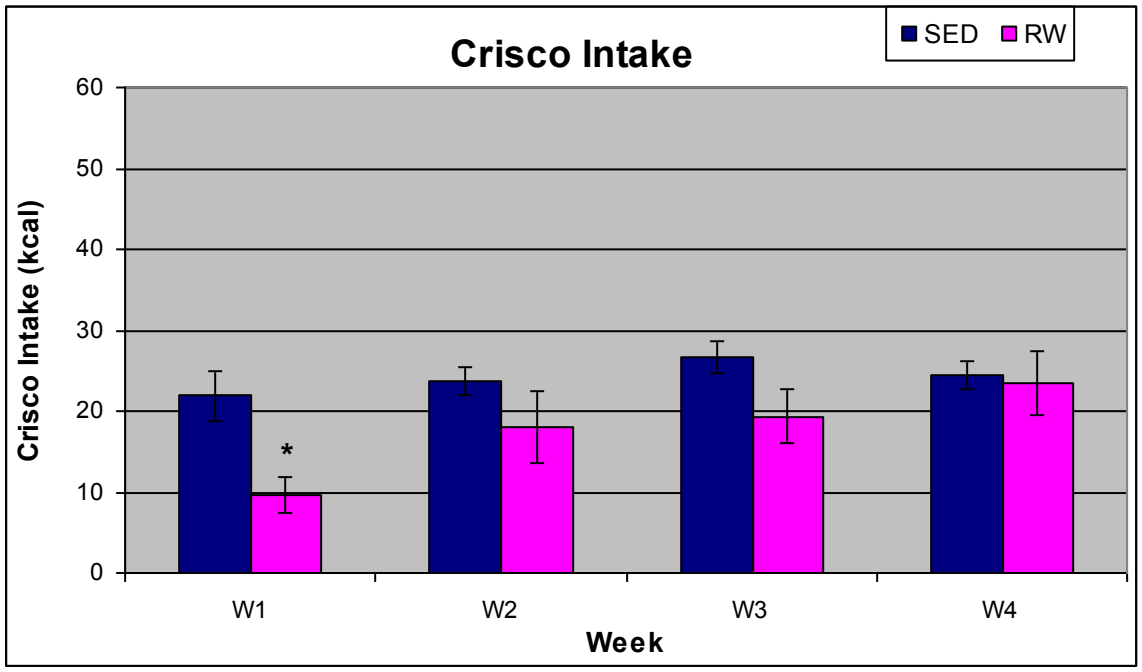

Figure 1. Average Crisco intake of the SED and RW groups across weeks one through four. During week one, the RW group ate significantly less Crisco than the SED group. (*$p<0.05$)

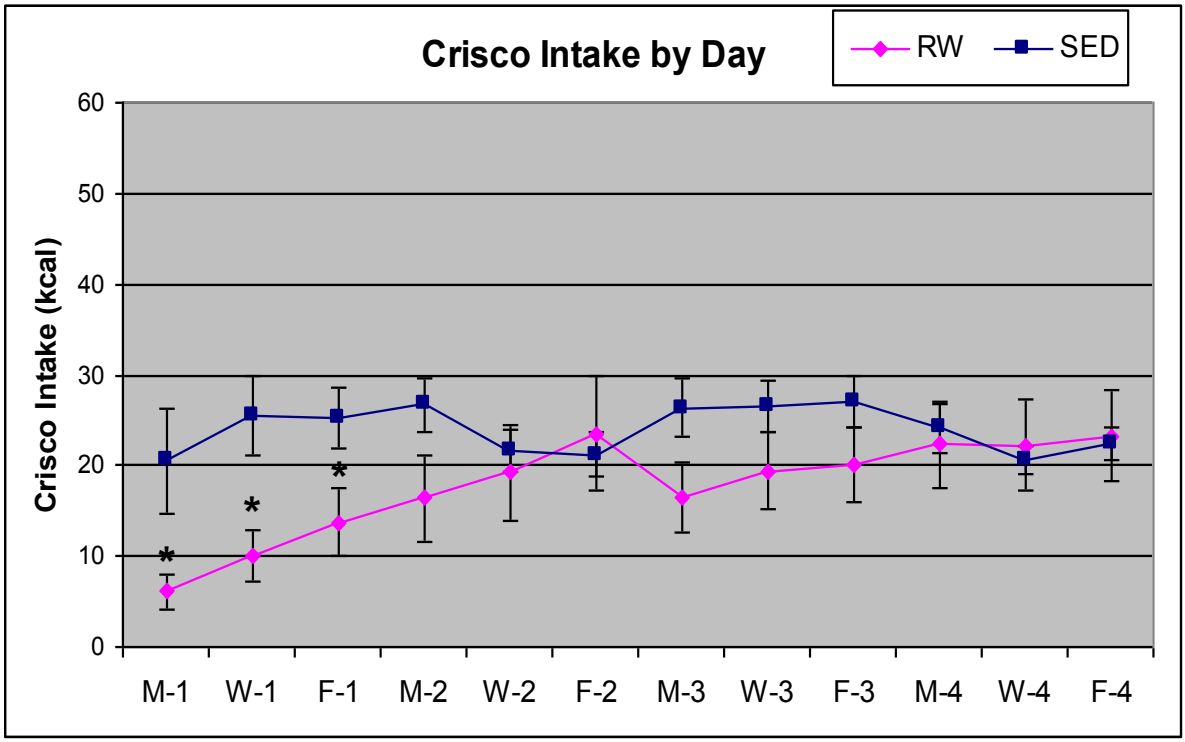

Figure 2. Average Crisco intake of the SED and RW groups for each Crisco access day (Mon., Weds., Fri.) across weeks one through four. During the Monday, Wednesday, and Friday of week one, the RW group ate significantly less Crisco than the SED group. (*$p<0.05$)

Within the BEP rats, there was no overall effect of RW access on Crisco intake ($F(1, 12) = 3.359$ $p=0.092$), but there was a significant time*group interaction ($F(3, 36) = 5.708$ $p=0.0027$) such that the RW-BEP rats began eating more Crisco than the SED-BEP rats by week four (Fig 3). The RW-BEP rats ate significantly more Crisco than the SED-BEP rats on the Weds. and Fri. of week two, but decreased their Crisco intake during week three, but rebounded and ate significantly more throughout week four (Fig 4).

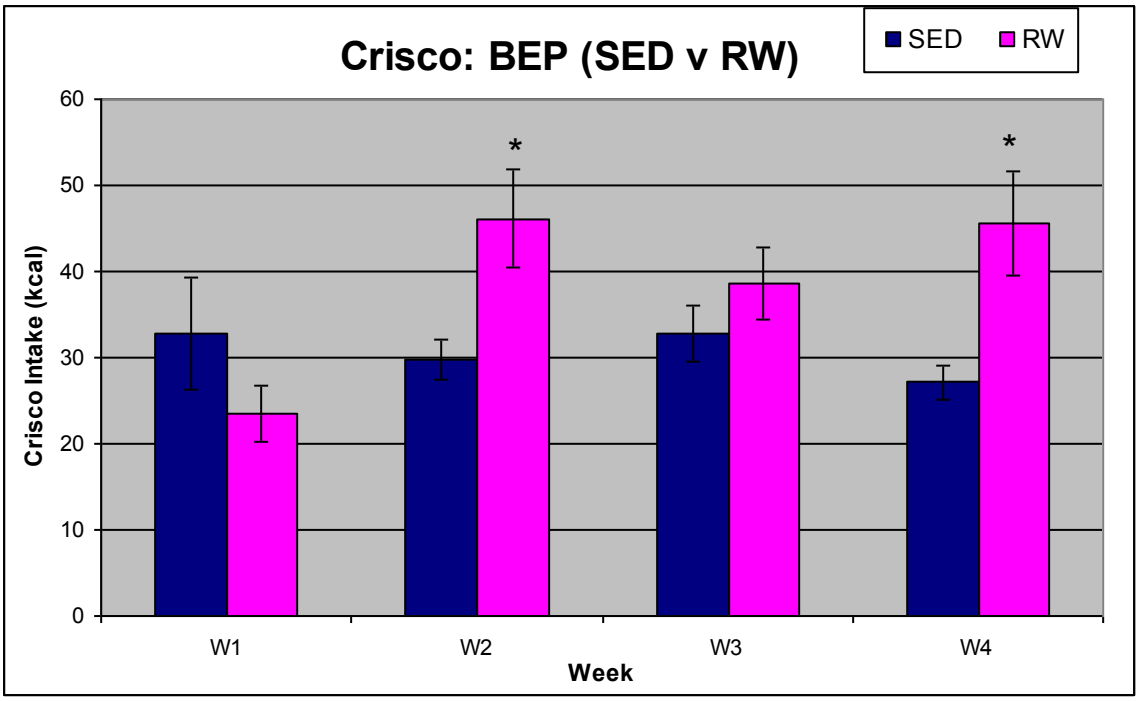

Figure 3. Average Crisco intake of all the BEP rats from both the SED and RW groups across weeks one through four. During weeks two and four, the RW-BEP rats ate significantly more Crisco than the SED-BEP rats. (*p<0.05)

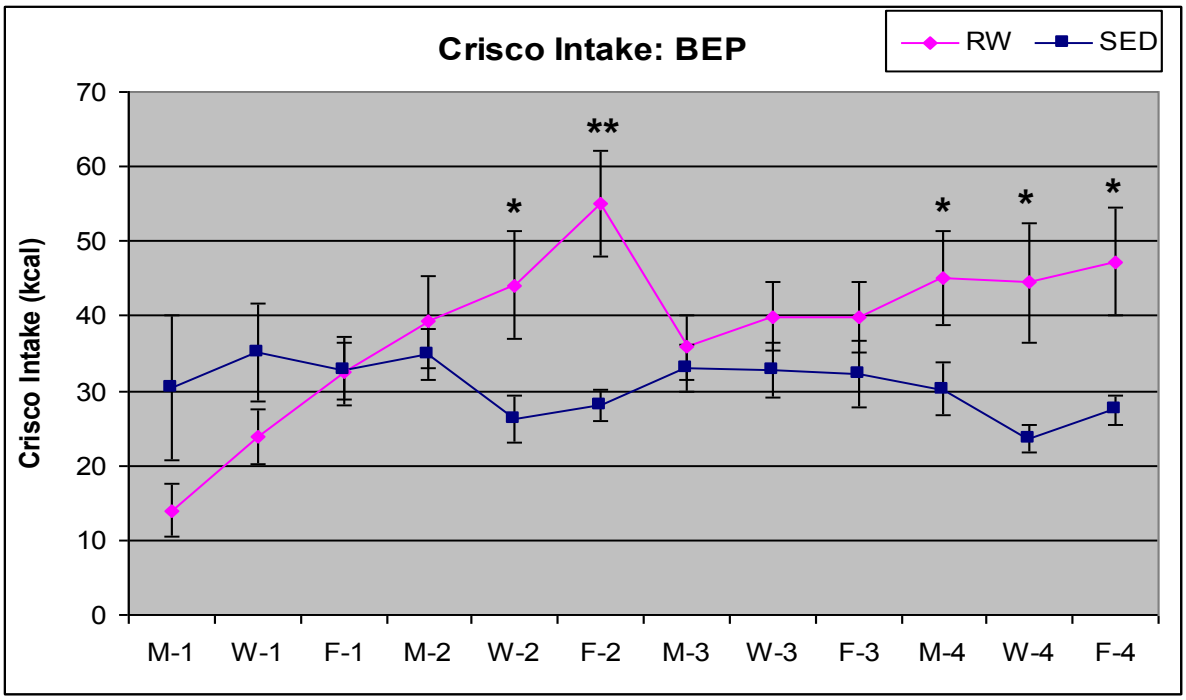

Figure 4. Average daily Crisco intake of all the BEP rats from both the SED and RW groups

for each Crisco access day (Mon., Weds., Fri.) across weeks one through four. On the Weds. and Fri. of week two and the Mon., Weds., Fri. of week four, the RW-BEP rats ate significantly more Crisco than the SED-BEP rats. (*p<0.05, **p<0.001)

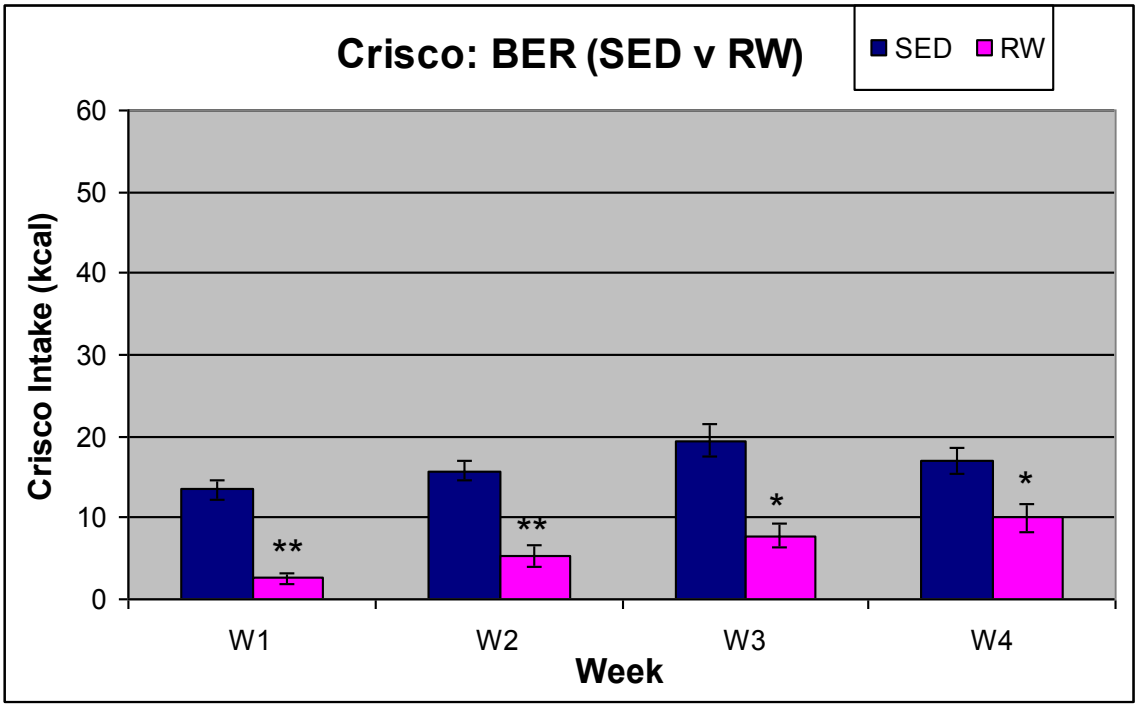

Figure 5. Average Crisco intake of all the BER rats from both the SED and RW groups across weeks one through four. During all four weeks, the RW-BER rats ate significantly less Crisco than the SED-BER rats. (*p≤0.05, **p<0.0001)

Within the BER rats, there was an effect of running wheel access on Crisco intake ($F(1, 16) = 38.264$ $p=0.0001$), whereby the SED-BER rats ate significantly more Crisco than the RW-BER rats (Fig 5). However there was no significant time*group interaction.

Within the Sedentary group, SED-BEP rats ate significantly more Crisco than the SED-BER rats ($F(1, 13) = 16.037$ $p=0.0015$). There was no significant time*binge interaction. Within the RW group, the RW-BEP rats ate significantly more Crisco than the RW-BER rats ($F(1, 15) = 181.86$ $p<0.0001$). There was also a significant time*binge interaction ($F(3, 45) = 5.226$ $p=0.0035$) such that weeks two through four the RW-BEP rats

consistently consumed large amounts of Crisco while the RW-BER rats only modestly increased their Crisco intake.

During the baseline measurements there were no significant differences in body weight ($p=0.74$), chow intake ($p=0.72$), or overnight Crisco intake ($p=0.92$) between the SED and RW groups. Once the rats were assigned to groups, prior to the start of the binge paradigm, the rats in the RW group weighed significantly less than the rats in the SED group ($p=0.0004$) (Fig 6). During the binge paradigm, there was an overall group effect ($F(1, 112) = 328.41$ $p<0.001$), such that the body weights of the rats in the RW group were significantly lower than those in the SED group (Fig 6). There was no significant time*group interaction. There was also an overall binge effect on body weight ($F(1, 112) = 9.74$ $p=0.002$), whereby the BEP rats weighed significantly more than the BER rats.

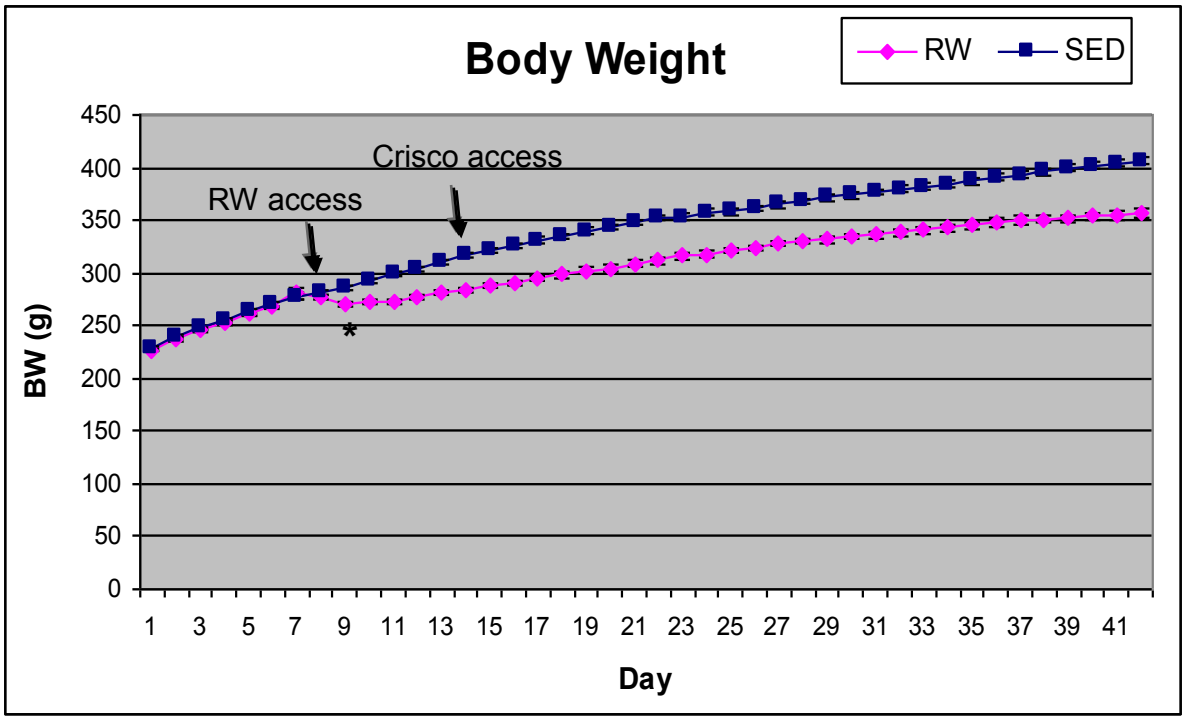

Figure 6. Average daily body weight of the SED and RW groups across the entire study. The first arrow marks where the running wheel access began (day 8). The second arrow marks

where the binge paradigm began (day 14). The RW group weighed significantly less than the SED group starting from the day marked with the asterisk (day 9) and continues throughout the experiment. (*$p<0.0001$)

Within the BEP group, the body weights of the RW-BEP rats were significantly lower than those of the SED-BEP rats ($F(1, 12) = 38.242$ $p=0.00005$). There was no significant time*group interaction. Within the BER group, the body weights of the RW-BER rats were significantly lower than those of the SED-BER rats ($F(1, 16) = 56.613$ $p<0.0001$). There was also a significant time*group interaction ($F(3, 48) = 6.85$ $p=0.0006$), whereby the SED-BER rats gained significantly more weight than the RW-BER rats over the four weeks. Within the sedentary group, there was no overall binge effect on body weight or time*binge interaction between the BEP and BER animals. Within the RW group, there was no overall binge effect, but there was a significant time*binge interaction ($F(3, 45) = 4.0968$ $p=0.01$) whereby the RW-BEP rats gained significantly more weight than the RW-BER rats over the four weeks.

The RW group ate significantly more chow than rats in the SED group ($F(1, 112) = 50.034$ $p<0.001$) (Fig 7 & 8). There was also a significant time*group interaction ($F(3, 112) = 4.334$ $p=0.006$), whereby there was no difference in chow intake at week one but from week two through four the RW rats ate significantly more chow than the SED rats (Fig 7). The daily chow intake shows a sawtooth pattern of consumption, whereby on days with Crisco access the rats ate less chow and on days without Crisco access the rats ate more chow, on average (Fig 8).There was an overall significant binge effect ($F(1, 112) = 89.65$ $p<0.001$), whereby the BER rats ate significantly more chow than the BEP rats. There was also a significant group*binge effect ($F(1, 112) = 44.475$ $p<0.001$).

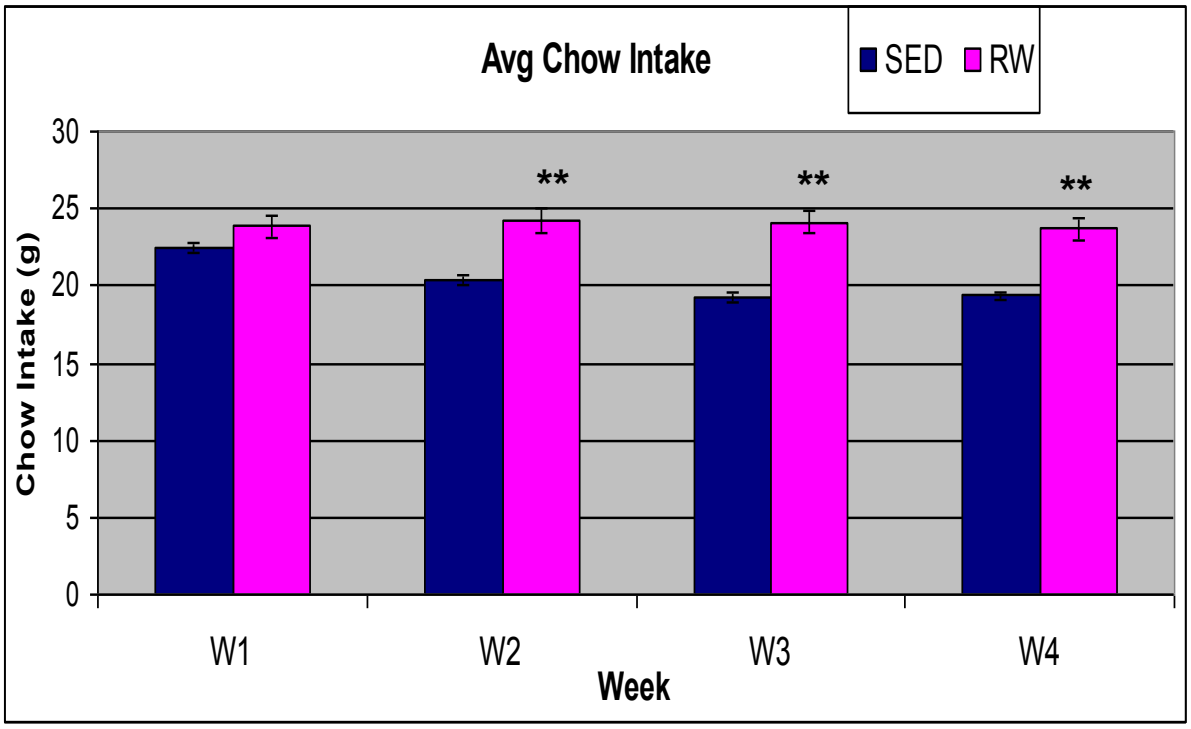

Figure 7. Average chow intake of the SED and RW groups across weeks one through four. During weeks two through four, the RW group ate significantly more chow than the SED group. (**p<0.0001)

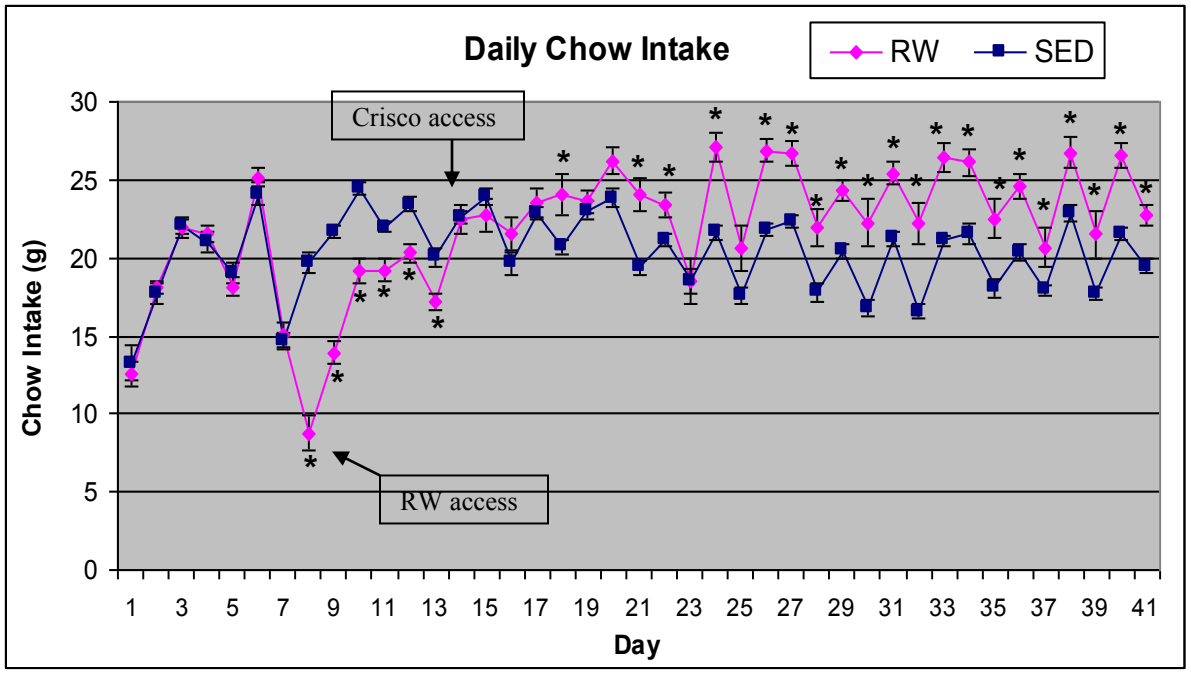

Figure 8. Average daily chow intake of the SED and RW groups across the entire study. The

RW rats ate significantly more chow than the SED rats on the days marked with asterisks. The first arrow marks where the running wheel access began (day 8). The second arrow marks where the binge paradigm began (day 14). (*$p<0.05$)

Within the BEP group, there was no overall group effect or time*group interaction on chow intake. Within the BER group, there was an overall group effect whereby the RW-BER rats ate significantly more chow than the SED-BER rats ($F(1, 16) = 36.405$ $p=0.0002$). There was also a significant time*group interaction ($F(3, 48) = 9.8996$ $p=0.00003$), whereby there was no difference in chow intake at week one but from week two through four the RW-BER rats ate significantly more chow than the SED-BER rats. Within the sedentary group, there was no overall binge effect or time*binge interaction on chow intake between the BEP and BER animals. Within the RW group, there was an overall binge effect ($F(1, 15) = 38.143$ $p=0.00002$) whereby the RW-BER rats ate significantly more chow than the RW-BEP rats. There was no significant time*binge interaction on chow intake between the BEP and BER animals.

There was an overall significant group effect on total caloric intake (chow + Crisco) ($F(1, 112) = 18.103$ $p<0.001$), whereby the RW group ate more overall than the SED group (Fig 9). This difference is most likely due to the high Crisco intake values of the RW-BEP rats. There was also a significant time*group interaction ($F(3, 112) = 11.73$ $p<0.001$), whereby on week one the SED group ate more than the RW group, but week two through four the RW group consumed more intake overall compared to the SED group (Fig 9). There was also a significant binge effect ($F(1, 112) = 70.35$ $p<0.001$), whereby the BEP rats ate significantly more overall than the BER rats.

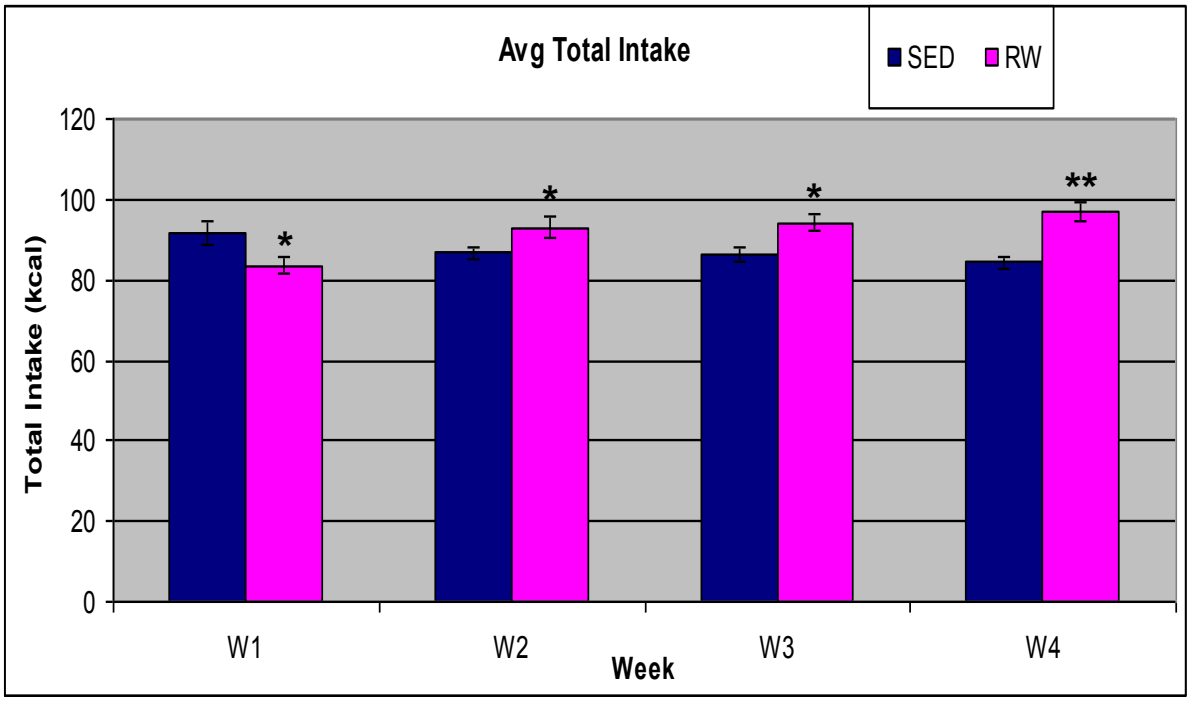

Figure 9. Average total intake of the SED and RW groups across weeks one through four. During week one, the RW group ate significantly less total intake (chow + Crisco) than the SED group. During weeks two through four, the RW group ate significantly more total intake than the SED group. (*$p \leq 0.05$, **$p < 0.0001$)

Within the BEP rats, there was no overall group effect, but there was a significant time*group interaction ($F(3, 36) = 16.348$ $p < 0.0001$), whereby there was no difference in total intake at week one, but from week two through four the RW-BEP rats consumed more total intake than the SED-BEP rats. Within the BER group, there was no overall group effect, but there was a significant time*group interaction ($F(3, 48) = 14.212$ $p < 0.0001$), whereby there was no difference in total intake at weeks one or two, but from week three through four the RW-BER rats consumed more total intake than the SED-BER rats. Within the sedentary group, there was an overall binge effect ($F(1, 13) = 10.112$ $p = 0.007$), such that the SED-BEP rats consumed significantly more total calories than the SED-BER rats. However, there was no significant time*binge interaction. Within the RW group, there was an overall binge effect

(F(1, 15) = 14.613 p=0.0017), whereby the RW-BEP rats consumed significantly more total intake than the RW-BER rats. There was also a significant time*binge interaction (F(3, 45) = 5.429 p=0.0028), whereby there was no difference in total intake at week one, but from week two through four the RW-BEP rats consumed more total calories than the RW-BER rats.

There was an overall binge effect on total running wheel activity (RWA) (F(1, 15) = 10.58 p=0.005), whereby the rats in the RW group that were classified as BER ran significantly more than those classified as BEP (Fig 10). This effect was consistent throughout the four week experiment, as there was no time*binge interaction.

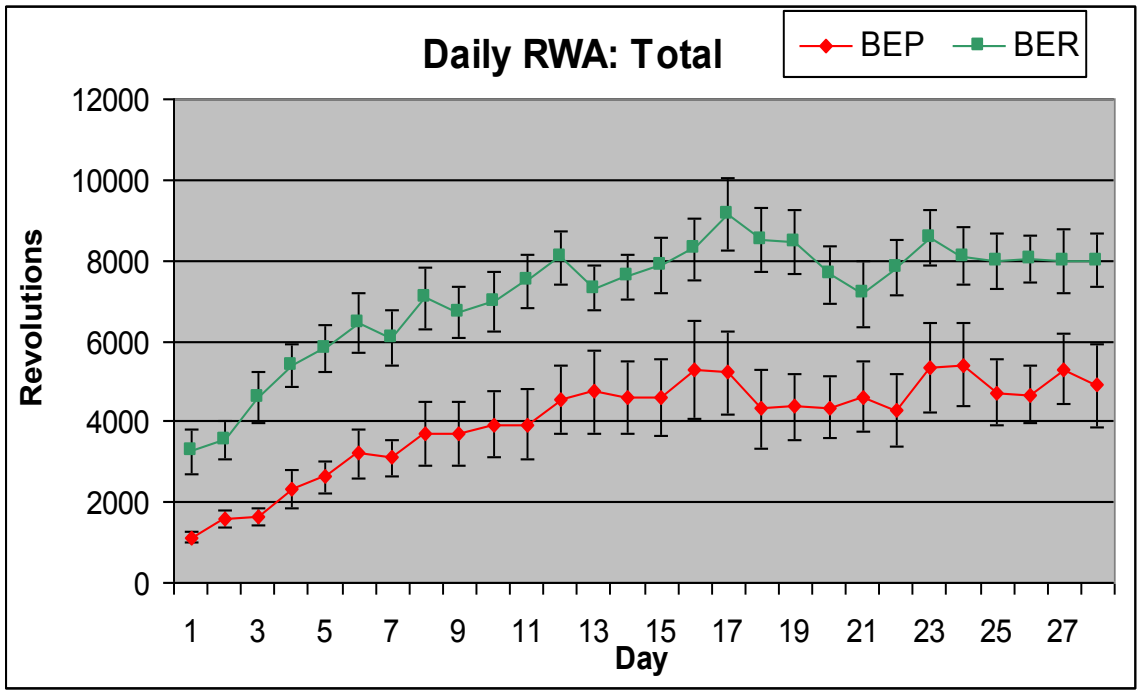

Figure 10. Average daily total running wheel activity (RWA) of the BEP and BER rats from the RW group across weeks one through four. Across the entire study, the RW-BER rats ran significantly more than the RW-BEP rats. ($p<0.05$)

There was also an overall binge effect on RWA during the dark cycle only (F(1, 15) = 11.11 p=0.0045), whereby the rats in the RW-BER rats ran significantly more than the RW-BEP rats. This effect was consistent throughout the four week experiment, as there was no

time*binge interaction. There was no binge effect on RWA during the light cycle only, but there was a significant time*binge interaction ($F(3, 45) = 5.13$ $p=0.004$) whereby during the first two weeks of the experiment the RW-BER rats ran more than the RW-BEP rats, but during the last two weeks, the RWA activity of the RW-BER and RW-BEP rats did not differ. There was also no binge effect on food-anticipatory activity (FAA), but there was a significant time*binge interaction ($F(3, 45) = 3.01$ $p=0.04$), whereby during the first week and a half the RW-BER rats ran more than the RW-BEP rats, but during the rest of the experiment both the RW-BER and RW-BEP rats ran similar amounts (Fig 11). Additionally, there was no binge effect on RWA during the one hour Crisco access period, but there was a significant time*binge interaction ($F(3, 45) = 4.34$ $p=0.01$), such that during the first two weeks the RW-BER rats ran more than the RW-BEP rats, but during the last two weeks both the RW-BER and RW-BEP rats ran similar amounts.

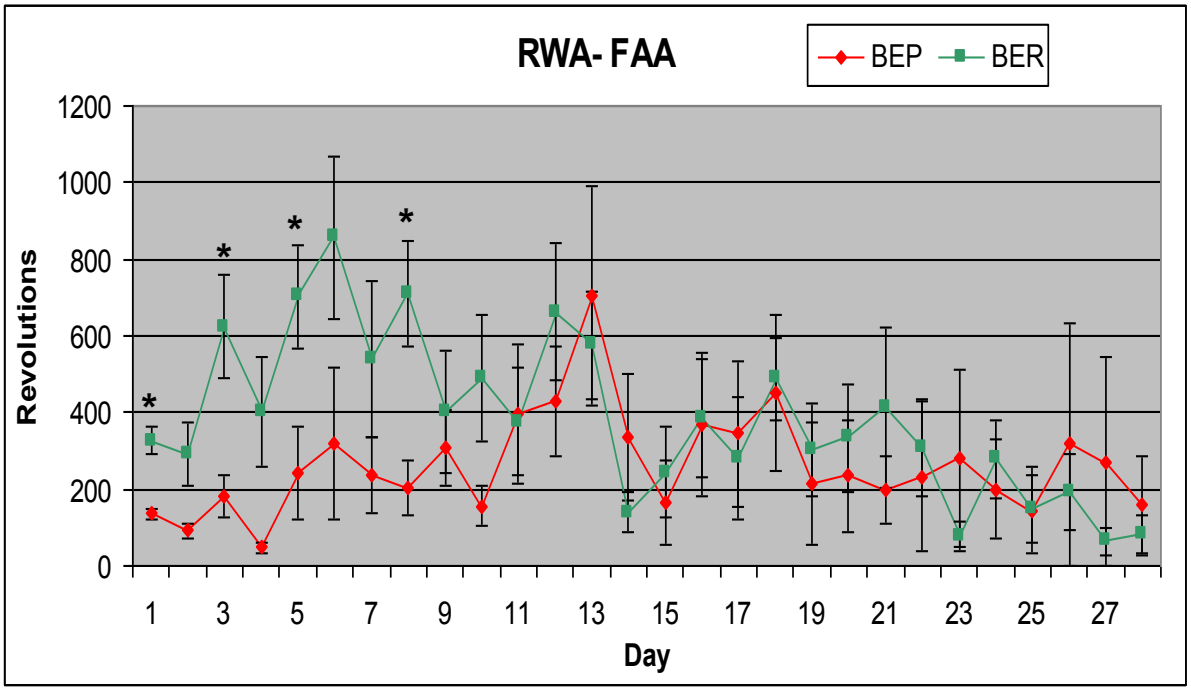

Figure 11. Average daily running wheel activity (RWA) during food anticipatory activity

(FAA) of the BEP and BER rats from the RW group across weeks one through four. The RW-BER rats ran significantly more than the RW-BEP rats on days 1, 3, 5, and 8. (*$p<0.05$)

We also found that there was a significant positive correlation of RWA with chow intake ($R^2= 0.76$), whereby those rats that ran the most also had the highest intake of chow (Fig 12).

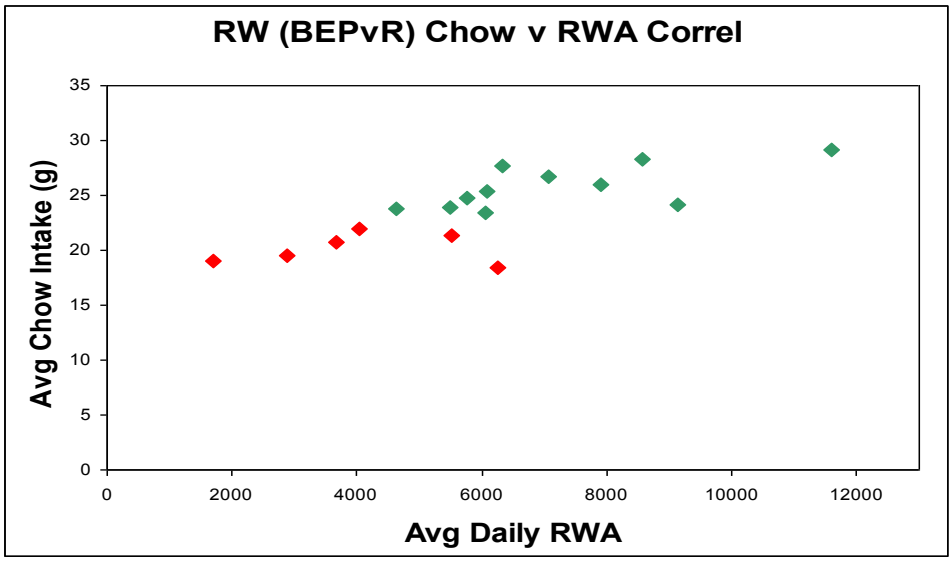

Figure 12. Scatterplot of the average daily running wheel activity (RWA) of the BEP and BER rats from the RW group against the average daily chow intake. There was a significant positive correlation of RWA with chow intake, whereby the rats that ran the most ate the most chow. Each dot represents an individual rat. The red dots represent the RW-BEP rats and the green dots represent the RW-BER rats. ($R^2= 0.76$, $p<0.05$)

Additionally, we found a significant negative correlation of RWA with Crisco intake ($R^2= -0.5$), whereby those rats that ran the most also had the lowest intake of Crisco (Fig 13). So overall, the rats that had the highest RWA also had the lowest Crisco intake and the highest chow intake.

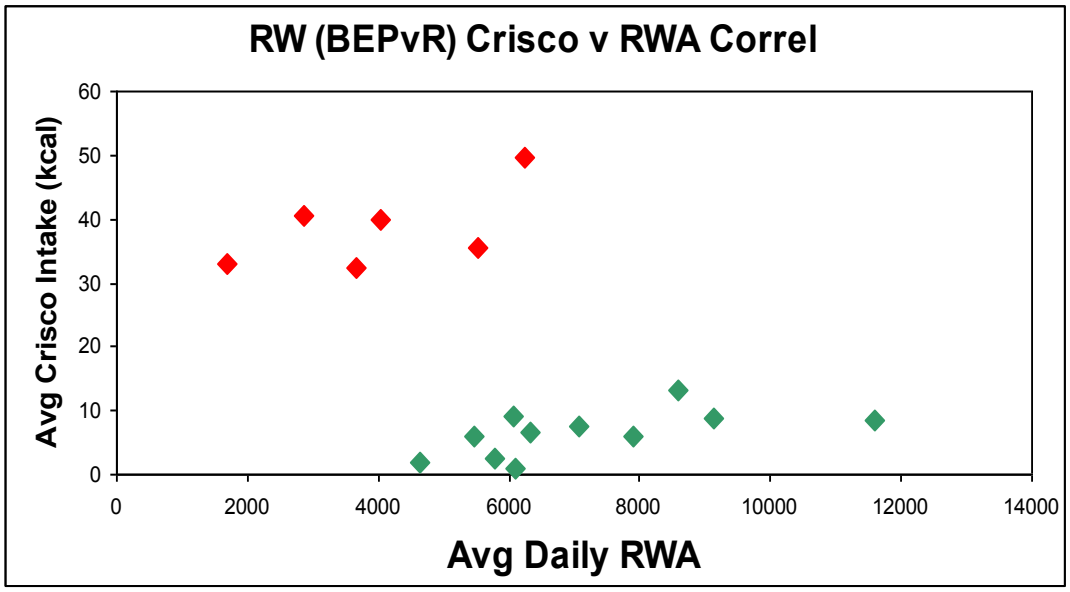

Figure 13. Scatterplot of the average daily running wheel activity (RWA) of all RW rats against the average Crisco intake. There was a significant negative correlation of RWA with Crisco intake, whereby the rats that ran the most ate the least Crisco. Each dot represents an individual rat. The red dots represent the RW-BEP rats and the green dots represent the RW-BER rats. (R^2= -0.5, $p<0.05$)

mRNA expression in the Ventral Tegmental Area

In the ventral tegmental area there were no significant differences among the groups on expression of the dopamine two receptor (D2R) or tyrosine hydroxylase (TH) (Table 1). Within the RW group, there was a significant negative correlation of TH expression with Crisco consumption, whereby the greater the Crisco consumption, the lower the expression (R^2= -0.5, $p\leq0.05$) (Fig 14).

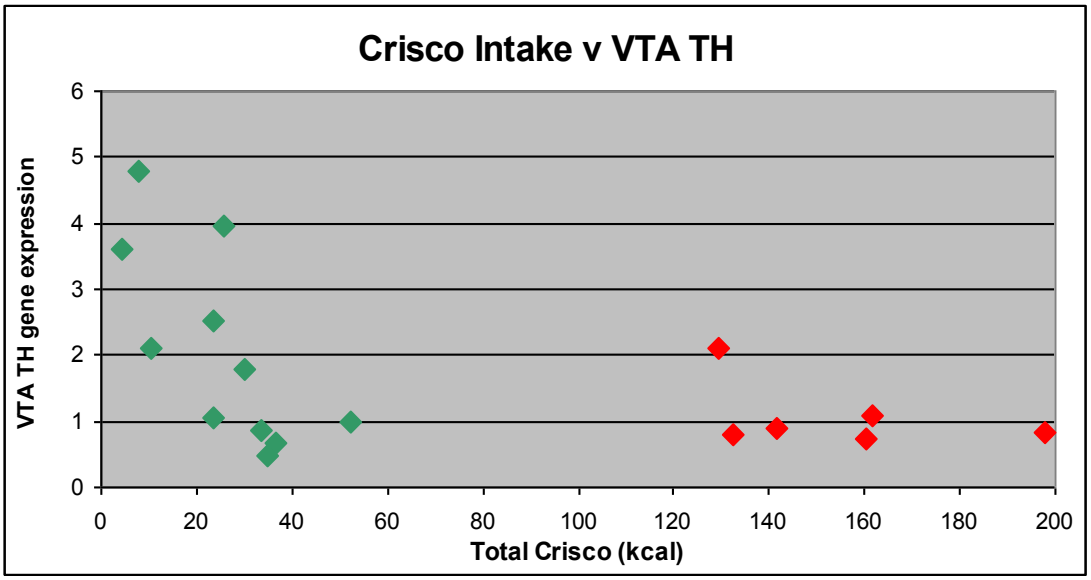

Figure 14. Scatterplot of the total Crisco intake of all RW rats against the fold change in gene expression of tyrosine hydroxylase (TH) in the ventral tegmental area (VTA). There was a significant negative correlation of Crisco intake with VTA TH expression, whereby the rats that ate the least Crisco displayed the greatest expression. Each dot represents an individual rat. The red dots represent the RW-BEP rats and the green dots represent the RW-BER rats. (R^2= -0.5, $p<0.05$)

Table 1. Dopaminergic mRNA gene expression in the ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC) of the SED-BEP, -BER, and RW-BEP, -BER rats, standardized to the SED-BER rats.

Group	SED		RW	
Binge status	BEP	BER	BEP	BER
TH in VTA	1.12 +/- 0.11	1.07 +/- 0.15	0.90 +/- 0.18	1.75 +/- 0.37
D2R in VTA	0.95 +/- 0.04	1.03 +/- 0.07	1.06 +/- 0.07	1.09 +/- 0.09
D2R in NAc	0.94 +/- 0.07	1.03 +/- 0.07	0.98 +/- 0.1	1.05 +/- 0.04
D2R in PFC	0.87 +/- 0.07	1.00 +/- 0.08	0.91 +/- 0.13	1.25 +/- 0.25

mRNA expression in the Prefrontal Cortex

In the prefrontal cortex there were no significant differences in expression of the D2R among the groups (Table 1). There were also no significant differences in the expression of the mu-opioid receptor (Oprm1) between the RW group and SED group, or between the BER animals and the BEP animals (Table 2). Within the RW group, there was no significant difference in the expression of the mu-opioid receptor (Oprm1) between the BER animals and BEP animals. However, within the SED group, there was a significant increase in the expression of the Oprm1 in the BEP animals compared to the BER animals (p=0.019) (Table 2).

Table 2. Opioid mRNA gene expression in the ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC) of the SED-BEP, -BER, and RW-BEP, -BER rats, standardized to the SED-BER rats. The Oprm1 expression in the NAc is significantly lower in the RW-BER rats in comparison to the RW-BEP (p=0.007) and SED-BER expression (p=0.025). The Oprm1 expression in the PFC is significantly higher in the SED-BEP rats in comparison to the SED-BER rats (p=0.019).

Group	SED		RW	
Binge status	BEP	BER	BEP	BER
Oprm1 in NAc	0.96 +/- 0.08	1.01 +/- 0.07	1.02 +/- 0.05	**0.85* +/- 0.03**
Oprm1 in PFC	**1.19* +/- 0.04**	0.98 +/- 0.07	1.09 +/- 0.05	1.23 +/- 0.1

mRNA expression in the Nucleus Accumbens

In the nucleus accumbens there were no significant differences in expression of the D2R among the groups (Table 1). There were also no significant differences in the

expression of the Oprm1 between the RW group and SED group, or between the BER animals and the BEP animals (Table 2). Within the SED group, there was no significant difference in the expression of the mu-opioid receptor (Oprm1) between the BER animals and BEP animals. However, within the RW group, the expression of the Oprm1 in the BER animals was significantly lower than in the BEP animals ($p=0.007$) (Table 2). Additionally, the expression of the Oprm1 in the RW-BER animals was significantly lower than in the SED-BER animals ($p=0.025$). Within the RW group we also found a significant positive correlation of Crisco intake with Oprm1 expression ($R^2= 0.59$, $p\leq0.05$) (Fig 15).

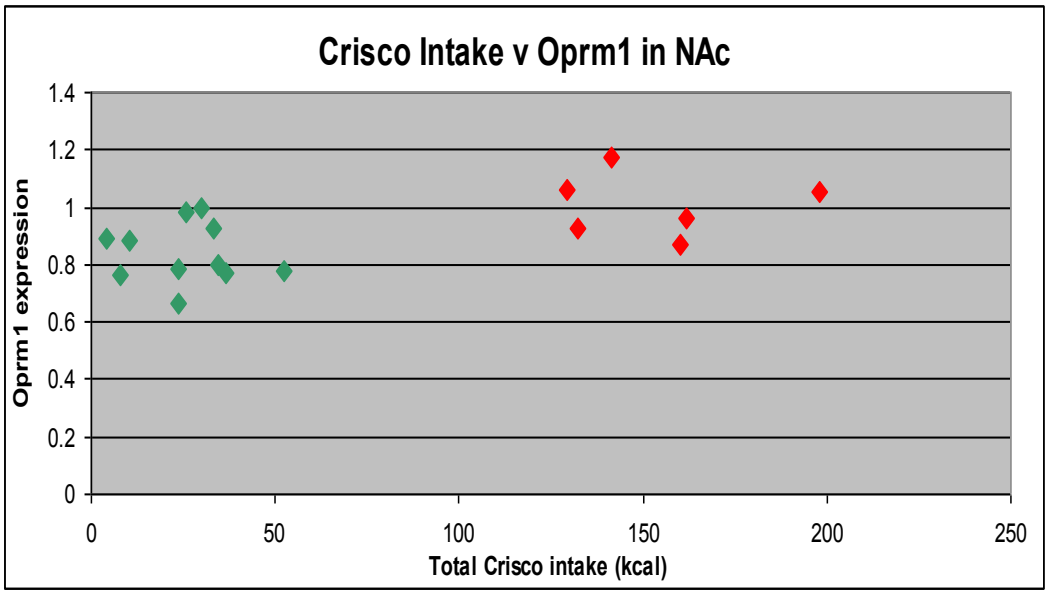

Figure 15. Scatterplot of the total Crisco intake of all RW rats against the fold change in gene expression of the mu-opioid receptor (Oprm1) in the nucleus accumbens (NAc). There was a significant positive correlation of Crisco intake with NAc Oprm1 expression, whereby the rats that ate the most Crisco displayed the greatest expression. Each dot represents an individual rat. The red dots represent the RW-BEP rats and the green dots represent the RW-BER rats. ($R^2= 0.58$, $p<0.05$)

Discussion:

The aim of this study was to investigate how voluntary running wheel access affects binge behavior within this limited access rat model of binge eating. Our data show that there was an overall effect of the running wheel (RW) on the first week of Crisco intake, but that was not maintained and by week two and throughout the rest of the study the intakes of Crisco were comparable between the RW and SED groups. On the first day of Crisco intake the RW group ate significantly less than the SED group, but over the first three days (week one) the Crisco intake of the RW group increased linearly, and by the Wednesday of week two the Crisco intakes were fairly equivalent between the two groups. While the Crisco intakes of the RW group tended to be a bit lower than the SED group, it was only significant during the first week.

Within this study the rats were categorized into groups based on their Crisco intake, ranging from binge-eating resistant (BER) to binge-eating prone (BEP) (10, 127). The BEP/BER model originated from the observation that, while rats of the same age and sex eat relatively equal amounts of standard laboratory rodent chow, their intake will vary when offered a highly palatable food (PF). This occurs when the PF is high in sugar and/or fat and is given intermittently (e.g., 2–3 times per week) to simulate the "forbidden" regard of these foods and diagnostic frequency in clinical binge eating (1, 127, 101, 118). Importantly, the expression of binge eating in the BEP rats is not observed until they first come in contact with PF (10, 127). That is, BEPs and BERs are indistinguishable until exposed to an environment containing PF. BEPs also do not have to learn to overeat PF; they overeat during the first exposure. Rats were ranked from low to high for 1 hour Crisco intake on each

of the first three binge days (Monday, Wednesday, and Friday of week 1). The rankings were combined across the three days and based on these rankings, rats were separated into sedentary BER (SED BER), sedentary BEP (SED BEP), RW BER, and RW BEP. These groups remained consistent throughout the four week study such that the rats categorized as BEP consistently consumed more Crisco than the rest of the rats and the rats categorized as BER consistently consumed less Crisco than the rest of the rats. This protocol is similar to that used by other investigators in which rats are categorized into BEP/BER groups, whereby the kcal intake of PF of each of the rats is grouped into evenly distributed tertiles (127, 128, 185, 101, 124). Similarly, it has been shown that Wistar rats on pure macronutrient diets, which exhibit a preference for fat, continue to show this preference throughout the full 4-week period of diet access. Thus, a tendency to overconsume a specific palatable diet with intermittent access is readily maintained and reliable (186).

Within both the SED and RW groups, the BEP rats consistently ate significantly more Crisco throughout all four weeks of the study than the BER rats. Boggiano developed a protocol for classifying binge status, whereby rats are given four feeding tests which involve giving rats a preferred food (PF) (Oreo cookies) and chow, and measuring intake after 4 h (127). These feeding tests are separated by at least 1 day of ad libitum chow only, so as to impose some form of sporadic access. For each of the four feeding tests, the PF kcal intake of each of the rats is grouped into tertiles. That is, the values and their corresponding rat identification are evenly distributed into three groups: a lowest, middle, and highest PF-intake group. Rats in the lowest PF-intake tertile across all four of the feeding tests (or in three out of the four) are assigned BER status. Those in the highest PF-intake tertile across

all four (or three out of the four) are assigned BEP status. How consistently a rat appears in a particular tertile is more important in determining status than the absolute kcal value of PF consumed. There is no specific amount of kilocalories that determine BEP or BER status. As pointed out by Klump et al., this also parallels the method by which clinical binge-eating was first defined; it was based on comparing women in the high vs. low ends of the binge eating distribution (1, 17, 128). Once rats with an inherent penchant to do this (BEPs) are identified from those that do not (BERs), it was discovered that there are many more behavioral parallels to human binge eating than just eating abnormally large amounts of food in discrete periods of time (127). Like clinical binge-eating, BEPs binge in the absence of hunger, the binges override satiety, PF intake remains high under stress, BEPs tolerate aversive consequences in order to obtain PF, and only some BEP rats are prone to obesity.

Within the BEP rats, we can see that the RW-BEP rats initially ate less Crisco during week one of the binge paradigm, consuming significantly less Crisco on day one. However, the RW-BEP rats began progressively eating more Crisco, eventually exceeding the steady amounts consumed by the SED-BEP group. Within the BER rats from both the RW and SED groups, we can see that the RW-BER rats consistently ate significantly less Crisco than the SED-BER rats. Thus, it appears that there is a lasting effect of exercise in the RW-BER rats, but not in the RW-BEP rats. Other studies have shown how diet choice can be altered upon exposure to a running wheel, oftentimes decreasing an animal's preference for a PF. In one study it was shown that rats introduced simultaneously to a sucrose solution and a running wheel engaged in running wheel activity but avoid consuming the sweet solution (93, 84). Additionally, when solid palatable food is provided (e.g. high fat (HF) diet) wheel running

can significantly reduce HF intake and thus decrease the preference of a previously preferred HF diet in a two-diet (standard chow vs. HF) choice feeding schedule (91, 92). In another study in which wheel running access was initiated a few days before the access to a novel HF diet, rats initially showed hyperphagic responses to the HF diet. However, HF diet intake gradually declined and the rats ended up showing no preference between the HF and standard chow diets. The current study is unique in that the rats did not have continuous access to the HF diet in addition to running wheel access, but rather had sporadic, limited access to a HF diet. In this study, the running wheel activity had differential effects on the rats depending on their propensity to binge eat.

Prior to dividing the rats into RW an SED groups they received overnight access to Crisco in order to avoid neophobia. After this overnight exposure, the rats were divided into RW and SED groups, with no significant differences in body weight, chow intake, or overnight Crisco consumption. We have retrospectively looked at the initial overnight Crisco intakes of the BEP and BER rats from both the RW and SED groups. Within the SED group, the overnight Crisco intakes correctly reflected the BEP and BER labels that they later received, with the rats that would later be categorized as BEP consuming more Crisco than those that would later be categorized as BER ($p=0.06$). However, within the RW group, the overnight Crisco intakes did not correctly reflect the BEP and BER labels that they later received, as the high and low Crisco intakes were scattered between both BEP and BER categories ($p=0.26$). Thus, we were unable to predict whether an animal was binge-eating prone or resistant after the initial overnight access to Crisco when the animal was exposed to a RW, as opposed to if the animal was sedentary (127). Additionally, although most groups

of rats, upon exposure to a highly palatable diet in an intermittent binge schedule, fairly equally distribute themselves into tertiles of BEP, BEN, and BER rats, this does not seem to be the case with rats exposed to a RW. What we see is that after exposure to the RW, a greater proportion of rats seem to become binge-eating resistant, with about twice as many rats being labeled BER in the RW group as those labeled BEP.

Across the four week study, the body weights of the RW rats were significantly lower than those of the SED rats. The RW rats also ate significantly more standard lab chow across weeks two through four compared to the SED rats. All the rats displayed a sawtooth pattern of chow intake, whereby they ate more chow on days without Crisco access and less chow on days with Crisco access as a way of compensating for the extra calories from Crisco. In studies by Corwin et al. where they also give rats sporadic, limited access to vegetable shortening, they also report seeing this binge/compensate pattern of feeding whereby rats overeat on days that palatable food is provided and undereat when palatable food is not provided (124). In studies by Avena, Hoebel and colleagues in which rats had intermittent access to a sugar solution, rats were able to regulate their caloric intake as well and compensate for the excess calories acquired from sugar by eating less chow (136).

Several differences in gene expression were found within brain reward areas. While there were no overall differences between SED and RW groups or between BEP and BER rats, we did find some changes when we compared all four groups: SED-BEP, SED-BER, RW-BEP, and RW-BER. Within the ventral tegmental area (VTA), there was a trend for elevated tyrosine hydroxylase (TH), a marker for dopamine (DA), in the BER rats in the RW

group. Within the RW group, we also found a significant negative correlation of TH gene expression with Crisco intake, meaning that the less Crisco consumed the greater the TH expression. Thus, it appears as though the rats that are running the most and eating the least amounts of Crisco (RW-BER rats), show elevated TH expression, which could mean that they are experiencing reward not from bingeing but perhaps from running. In a study by Greenwood et al., it was demonstrated that six weeks of voluntary wheel running resulted in increased TH mRNA levels in the VTA of rodents (178). Running wheel activity has also been shown to be rewarding to rodents, and studies demonstrate that rats will lever-press for access to running wheels (71, 72, 73, 74, 75), similar to drug self-administration paradigms, which indicates that wheel running is also rewarding and rats will work for the opportunity to run. This rewarding aspect of voluntary wheel running suggests that neural pathways that regulate the reward experienced after drugs of abuse may also be activated by running wheel activity. This is supported by the finding that rats who exhibit the highest levels of lever-pressing also exhibit the highest levels of running wheel activity (73), suggesting that those rodents that receive the most reward from wheel running are also willing to work the hardest for it.

It has also been demonstrated that ingestion of palatable foods activates dopaminergic neurons within the VTA and other reward centers (100, 187, 188). Additionally, it is well established that addictive drugs activate DA containing neurons in areas of the brain that process reinforcement, and similar findings have been discovered in studies analyzing binge eating (112). However, upon repeated exposure, the dopamine response has been shown to habituate to a reward, whether it is drugs or food (5). Moreover, repeated stimulation of this

system in an attempt to relieve the physiological or psychological effects of stress or negative affect has been linked to the development of binge eating and substance abuse (189). Thus, there is the possibility that the rats, other than the RW-BER rats, have a dampening of TH release due to repeated stimulation from bingeing.

Furthermore, within the RW group, the mu-opioid receptor (Oprm1) expression in the NAc was significantly lower in the BER rats, relative to BEP rats. There was also a significant positive correlation of Oprm1 gene expression with Crisco intake in the RW group, meaning that the more Crisco consumed the greater the expression of Oprm1. Endogenous opioids within the NAc shell have been linked to the hedonic or reinforcing properties of food. Ingestion of sweet and fat foods and fluids increases opioid receptor binding within this region of the natural reward system (100, 193, 194). Data generated from the sugar addiction model has shown that sugar bingeing decreases enkephalin mRNA in the nucleus accumbens (124, 141), and mu-opioid receptor binding is significantly enhanced in the NAc shell compared with chow-fed controls (140). Also, the fact that sugar-bingeing rats are sensitive to the effects of the opioid antagonist naloxone, which can precipitate signs of withdrawal (145), suggests that repeated bouts of excessive sugar intake can alter brain opioid systems. Although our model did not utilize sugar, we still found a positive correlation between highly palatable diet intake and the mu-opioid expression within the NAc, which is consistent with the literature. Results from the history of dieting (HD) + stress and limited access models also lend support for a role of opioids in binge eating behavior. HD + stress induced binge-eating is abolished by naloxone, a mixed kappa/mu opioid receptor antagonist (105, 100). Similarly, nalmefene, a mu and kappa opioid antagonist, significantly attenuated

binge eating in rats conditioned to binge eat a chocolate flavored, high sugar diet (100). Likewise, rats given intermittent access to a 25% glucose solution for 30 days showed increased opioid receptor binding in the nucleus accumbens shell (140, 100).

Within the PFC, we saw a significant increase in Oprm1 expression in the SED-BEP rats, relative to the SED-BER rats. This finding is consistent with a study by Blasio et al., in which mPFC microinfusions of an opioid antagonist (naltrexone) selectively and dose-dependently decreased both the consumption and the motivation to obtain a highly palatable diet in binge eating rats, without affecting the intake of regular chow in control rats (195). Water intake was not affected by drug treatment in either group, suggesting that the effects are selective for feeding behavior. Altogether, the results of Blasio's study support the hypothesis that the opioid system in prefrontal cortical regions of the brain is involved in the control of feeding behavior and expand it to the specific context of the maladaptive excessive intake of highly palatable food observed in binge eaters (196, 195). Frontal cortical regions of the brain play a major role in reward evaluation and decision making (197, 195), and a vast body of literature demonstrates that subjects afflicted by addiction and binge eating disorder show dysfunctions in the mPFC, which are associated with altered reward evaluation (198, 195).

In summary, we found that access to a running wheel did not decrease bingeing behavior overall, which was contrary to our initial hypothesis. Within the binge-eating resistant rats, the running wheel activity decreased Crisco intake and binge behavior, but within the binge-eating prone rats, the running wheel activity did not help to decrease

bingeing behavior. We found various alterations in the dopaminergic and opioid reward pathways within the prefrontal cortex, nucleus accumbens, and ventral tegmental areas of both the sedentary and running wheel access rats. Thus, it seems that both bingeing as well as running stimulates these reward pathways in the brain and plays a role in modifying eating behavior. One of the questions here regards the relationship between exercise and the binge status of the rat (BEP or BER). Is it that an individual runs more and thus they tend to be binge-eating resistant? Or is it that the individual is not bingeing and thus increases his/her activity? More research will have to be done in order to tease apart these distinctions. Furthermore, adding to the clinical relevancy, it would be very interesting to see if sedentary rats that had already developed a bingeing phenotype were able to decrease their Crisco intake and stop bingeing upon exposure to a running wheel. Additionally, it would be interesting to know if previous exposure to a RW would help to prevent bingeing in rats that became sedentary. Consequently, these are two of the questions that I planned on examining in future experiments.

Chapter 4: Experiment 2- Effects of Running Wheel Activity on Established Binge Eating

Abstract:

Binge eating disorder (BED) is an eating disorder involving repeated, intermittent over consumption of food in brief periods of time, usually with no compensatory behaviors. There are few successful treatments and the underlying neural mechanisms remain unclear. In the current study, we hypothesized that voluntary running wheel (RW) activity could reduce binge eating behavior in a rat model. Rats were given sporadic (3 times/wk) limited (1hr) access to a high-fat food (Crisco), in addition to continuously available chow. Crisco was available every Mon, Wed, and Fri for 1hr before dark onset. Rats were divided into 2 groups: those with RW access during the first half of the experiment and sedentary during the second half (RW-SED) and those that were sedentary during the first half of the experiment and had RW access during the second half (SED-RW). Crisco intake was significantly less in both groups during the period of time with a RW present. Within the bingeing RW-SED rats, the gene expression of the orexigenic neuropeptides AgRP and NPY were similar to a non-bingeing sedentary control (CON) group, while the expression of the anorexigenic neuropeptide POMC was significantly increased relative to the SED-RW and CON groups. Despite elevated POMC, the rats continued to binge. Additionally, within both groups, the gene expression of the D2R and Oprm1 in the NAc and VTA suggested that the reward system was stimulated by both the bingeing behavior as well as the running wheel activity. Overall, access to a RW and the resulting activity significantly reduced binge behavior as well as modulated the effects of binging on brain appetite and reward systems.

Introduction:

In a previous study performed in our lab, we found that access to a running wheel initially significantly decreased binge eating behavior in rodents, but within two weeks the Crisco intake increased up to the levels of sedentary controls. Based on week 1 Crisco intake, both groups were divided into Binge Eating Prone (BEP) and Resistant (BER) animals. We found that running wheel activity (RWA) significantly decreased binge-eating in the resistant rats but not in the prone rats. These results were not consistent with our overall hypothesis in that RW access did not decrease Crisco intake in the BEP rats. We also found a significant increase in PFC Oprm1 mRNA in the SED-BEP group relative to SED-BER, as well as decreased NAc Oprm1 mRNA and a trend toward increased TH mRNA expression in the VTA of the RW-BER rats. Overall, access to a RW and the resulting activity reduced binge behavior in the RW-BER animals as well as modulated the effects of binging on brain reward systems. However, within this study, the rats had access to running wheels just prior to the start of the binge paradigm. Therefore, in order to examine a more clinically relevant question, we wanted to investigate the effects of running wheel activity on established bingeing behavior.

In the current experiment we investigate how scheduled running wheel access affects binge behavior within this limited access rat model of binge eating. We designed the experiment to assess whether running wheel access and the resulting activity would prevent binge behavior from developing and whether it would reduce binge behavior after bingeing had been established. To do this, one group (SED-RW) was sedentary during the first three weeks of the experiment, and then once bingeing had been established, they were provided

with RW access during the last three weeks of the experiment. We also wanted to assess whether RW access would help to prevent rodents from bingeing by providing them with RW access prior to the establishment of binge behavior. To do this, one group (RW-SED) had RW access during the first three weeks of the experiment, prior to the establishment of bingeing, and then was sedentary during the last three weeks of the experiment. We hypothesize that voluntary running wheel activity would reduce binge eating behavior in rats. In order to evaluate the mechanisms underlying vulnerability to binge eat, we measured gene expression of several genes involved in appetite, food reward, and reinforcement, including: tyrosine hydroxylase (TH), the mu-opioid receptor (Oprm1), the dopamine-2 receptor (D2R), the agouti-related peptide (AgRP), neuropeptide Y (NPY), Pro-opiomelanocortin (POMC), and the leptin receptor (LepR). We analyzed these genes within various brain reward and appetite regions, including: the prefrontal cortex (PFC), the nucleus accumbens (NAc), the lateral hypothalamus (LH), the arcuate nucleus (ARC), and the ventral tegmental area (VTA). These genes (TH, Oprm1, D2R) were chosen because in other rodent studies it has been shown that binge-eating is able to activate the reward system, which typically involves the modulation of dopamine and endogenous opioid signaling (113, 114, 213, 101, 124). Additionally, human imaging studies have shown that individuals with binge eating disorder may have impairments in dopaminergic pathways that regulate neuronal systems associated with reward sensitivity, conditioning, and control (5). The other chosen genes (AgRP, NPY, POMC, LepR) are involved in the regulation of appetite and activity (222).

Methods:

Animals

A total of forty-two adult male Sprague Dawley rats from Harlan we obtained when they were between fifty to sixty days old and weighed 220-240g. They were individually housed in plastic tub cages in a temperature- and humidity-controlled room under a 12:12-h light-dark schedule (lights off from 12PM to 12AM). All rats had ad libitum access to water and standard laboratory rodent chow (Harlan 2018, Fredrick, MD; 3.1 kcal/g: fixed formula diet of 18.6% protein, 44.2% carbohydrate, and 6.2% fat). All animal procedures were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine.

Experimental set-up

All rats had unlimited access to the standard laboratory rodent chow diet and water throughout the study. At the end of the 1-week adaptation period, seven days prior to week one Crisco access, all rats were given overnight access to a jar of hydrogenated vegetable shortening (Crisco® brand All-Vegetable Shortening, J.M. Smucker Company), clipped to the front of the cage. Rats were then divided into two groups: those with RW access during the first half of the experiment and sedentary during the second half (RW-SED) (n=21), and those that are sedentary during the first half of the experiment and have RW access during the second half (SED-RW) (n=21), with no significant mean differences in baseline overnight Crisco intake, chow intake, or body weight. Additionally, five days prior to week one Crisco access, the RW-SED group began habituating to their running wheels and continued to have unlimited running wheel access during the first three weeks of the

experiment. After Crisco access on the Friday of week 3, the RW-SED rats were moved to cages without running wheels, and the SED-RW group was moved from sedentary cages to cages with running wheels and habituated to the wheels for the remainder of that Friday and through the Saturday and Sunday of week 3, and remained in the RW cages for the remaining three weeks of the experiment. Throughout these six weeks, rats were given sporadic (3 times/wk) limited (1h) access to a high-fat food (Crisco®). Crisco was available every Monday, Wednesday, and Friday during the last 1h of the light cycle, immediately preceding the onset of the dark cycle. Another group of sedentary control rats, not given access to Crisco and body weight matched to the RW-SED and SED-RW groups, was also used as controls for the gene expression analyses (CON, n=8).

One-hour and 24-h intakes of shortening and standard chow, respectively, were measured every day throughout the study by weighing the shortening and standard diet and subtracting the current food weight from the most recent previous food weight. Body weights were measured daily. Running wheel activity (RWA) was also recorded daily using a cage registration program (VitalView, Mini Mitter, Bend, OR). RWA was assessed during various time periods: the light cycle, dark cycle, total daily RWA, during the 1h Crisco access time period, and during the food-anticipatory period (FAA). FAA is an increase in locomotor activity preceding the presentation of food (specifically Crisco in this case) that develops when food is available during a restricted and predictable time of the day (179). In this study, FAA was classified as the RWA during the three hour period prior to the Crisco access.

On the Monday of week 7, all rats were decapitated 1h prior to regularly scheduled Crisco access. The brains were collected and stored at -80°C. Brain punches were taken of the Prefrontal Cortex (PFC), Nucleus Accumbens (NAc), Ventral Tegmental Area (VTA), Arcuate Nucleus (ARC), and Lateral Hypothalamus (LH), and RT-PCR was performed to determine mRNA expression.

mRNA expression

The Prefrontal Cortex (PFC), Nucleus Accumbens (NAc), Ventral Tegmental Area (VTA), Arcuate Nucleus (ARC), and Lateral Hypothalamus (LH) were isolated from 500 μm thick frozen coronal sections using a Harris uni-core tissue puncher (ID 0.75 mm) (Ted Pella, Inc., Redding, CA, USA) based on the coordinates for adult rat brains (180). The RNeasy Lipid Tissue Mini Kit with the Qiazol reagent (Qiagen, Valencia, CA) was used to isolate total RNA from the tissue punches. 200ng of RNA was used to generate cDNA for subsequent quantitative real-time PCR with a QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA). All reactions were carried out in triplicate using 1X Taqman master mix (Applied Biosystems, Foster City, CA), cDNA template, and 1X Taqman probes for each gene (Oprm1, D2R, TH, AgRP, POMC, NPY, LepR, Orexin, and Actb) (Life technologies, Grand Island, NY) in a total volume of 20μL. Real-time reactions were performed with standard PCR conditions (50°C for 2 min; 95°C for 10 min; and 95°C for 15 sec and 60°C for 1 min for 40 cycles) on an Applied Biosystems 7900HT Fast Real-Time PCR System. Each set of triplicates was checked to ensure that the threshold cycle (Ct) values were all within 1 Ct of each other. Negative RT samples were used to control for possible contamination of cDNA. To determine relative expression values, the -ΔΔCt method

(Applied Biosystems, Foster City, CA) was used, where triplicate Ct values for each tissue sample were averaged and subtracted from those derived from the housekeeping gene Beta-actin (Actb). The average Ct difference for the control group (sedentary rats with no Crisco access, CON) was subtracted from those of the test samples, and the resulting -ΔΔCt values were raised to a power of two to determine normalized relative expression.

Plasma leptin analysis

At sacrifice, trunk blood (3ml) was collected into heparinized micro-centrifuge tubes, centrifuged at 4°C to collect plasma, and plasma was stored at -80°C for leptin analysis. Leptin levels in the plasma were analyzed using a commercially available Leptin Elisa kit (Millipore, Billerca, MA). Inter- and intra- assay variability, respectively, were as follows: 2.95–3.93% and 1.88-2.49%.

Statistical Methods

Data are presented as means ± standard error of the mean. Statistical analyses were performed using Statistica 7 (Systat, Tulsa, OK) and SigmaPlot 11.0 (Systat Software Inc) software. Group differences in body weight, chow intake, Crisco intake, total intake, Crisco/Total intake, and running wheel activity were assessed with a mixed model repeated measures ANOVA with running wheel exposure (GROUP) as the between subjects factor and time as the repeated factor. Differences at specific time points and between specific experimental groups were assessed by one-way ANOVAs and Tukey's post-hoc tests. Group differences in mRNA expression were also assessed by one-way ANOVAs and Tukey's post-hoc tests. For all statistical analysis a confidence interval of 95% was used.

Results:

Crisco Intake, Body weight, Chow intake, Total intake, Crisco/Total intake, and running wheel activity

There was an overall significant group effect on Crisco intake over the entire six week study ($F(1, 240) = 5.47$ $p=0.02$), whereby the RW-SED group ate significantly more Crisco overall in comparison to the SED-RW group (Fig 16). There was also a significant time*group interaction ($F(5, 240) = 15.262$ $p<0.0001$), whereby over time the Crisco intake of the groups switched, and the group eating more Crisco during the first three weeks (SED-RW) began eating less Crisco in the last three weeks, and vice versa, due to the switching of the running wheel access (Fig 16). Analyses of Crisco intake during the first three weeks, when the SED-RW group was sedentary and the RW-SED group had RW access, showed that the SED-RW group ate significantly more Crisco than the RW-SED group ($F(1, 120) = 19.09$ $p<0.001$). Also, during the last three weeks when the RW-SED group was sedentary and the SED-RW group had RW access, the RW-SED group ate significantly more Crisco than the SED-RW group ($F(1, 120) = 64.09$ $p<0.001$). Additionally, there was no significant time*group interaction within either the first three weeks ($F(2, 120) = 0.083$ $p=0.921$) or within the last three weeks ($F(2, 120) = 0.424$ $p=0.656$).

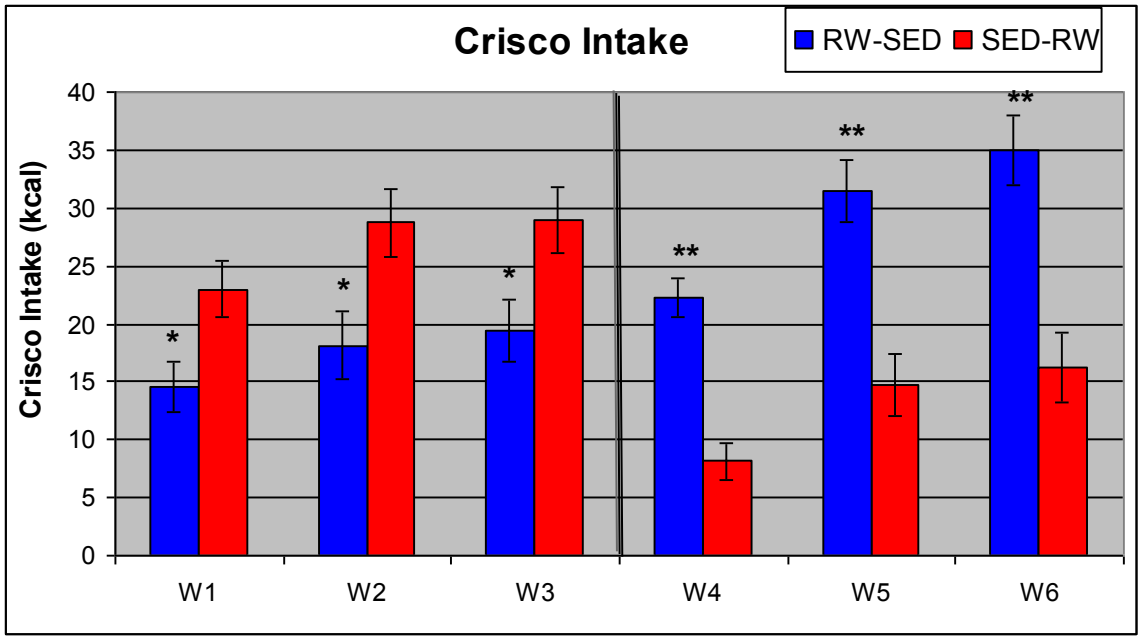

Figure 16. Average Crisco intake of the RW-SED and SED-RW groups across weeks one through six. During weeks one through three, the RW-SED group (RW access) ate significantly less Crisco than the SED-RW group (sedentary). During weeks four through six, the RW-SED group (sedentary) ate significantly more Crisco than the SED-RW group (RW access). (*$p<0.05$, **$p<0.0001$)

During the baseline measurements there were no significant differences in body weight ($p=0.73$), chow intake ($p=0.55$), or overnight Crisco intake ($p=0.67$) between the RW-SED and SED-RW groups. During the binge paradigm there was an overall group effect over the entire six week study in which the body weights of the rats in the SED-RW group were significantly greater than those in the RW-SED group ($F(1, 240) = 21.05$ $p<0.001$) (Fig 17). There was also a significant time*group interaction ($F(5,240) = 11.008$ $p<0.001$), whereby the body weights of the SED-RW group decreased during the 2nd 3 weeks while the weights of the RW-SED group increased (Fig 17). During the first three weeks when the SED-RW

group was sedentary and the RW-SED group had RW access, the SED-RW group weighed significantly more than the RW-SED group ($F(1, 120) = 99.98$ $p<0.001$). During the last three weeks when the RW-SED group was sedentary and the SED-RW group had RW access, there was no main effect of body weight ($F(1, 120) = 1.54$ $p=0.216$). However, there was a significant time*group interaction ($F(2, 120) = 3.93$ $p=0.022$) whereby the body weights of the SED-RW group stabilized while the body weights of the RW-SED group continued to increase.

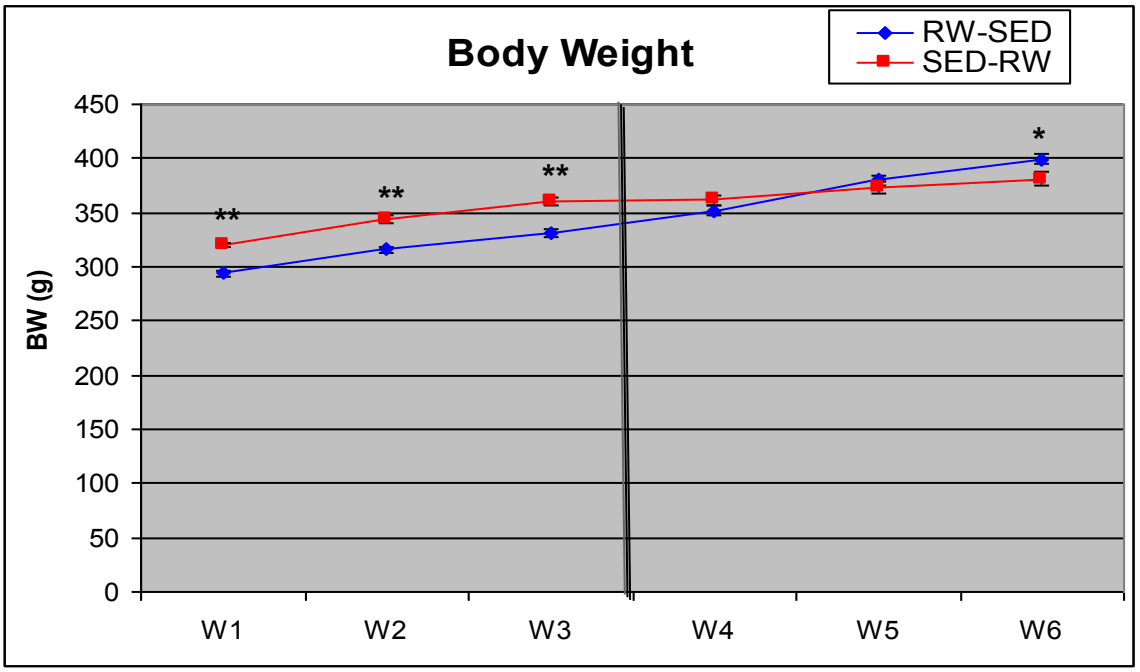

Figure 17. Average body weight of the RW-SED and SED-RW groups across weeks one through six. During weeks one through three, the RW-SED group (RW access) weighed significantly less than the SED-RW group (sedentary). By week six, the RW-SED group (sedentary) weighed significantly more than the SED-RW group (RW access). (*$p<0.05$, **$p<0.0001$)

Over the entire six weeks, there was a significant group effect on total chow intake ($F(1, 240) = 12.92$ $p<0.001$), whereby the RW-SED group ate significantly more chow than the SED-RW group overall (Fig 18). There was also a significant time*group interaction ($F(5,240) = 38.94$ $p<0.001$) whereby during the 2nd 3 week period the RW-SED group ate significantly less chow and the SED-RW group ate significantly more chow (Fig 18). This is demonstrated by the results of the separate ANOVA's for the two 3 week periods. During the first three weeks when the SED-RW group was sedentary and the RW-SED group had RW access, the RW-SED group ate significantly more chow than the SED-RW group ($F(1, 120) = 108.47$ $p<0.001$). There was also a significant time*group interaction ($F(2, 120) = 7.65$ $p<0.001$) whereby over the first three weeks the SED-RW group ate significantly less chow relative to the RW-SED group. During the last three weeks when the RW-SED group was sedentary and the SED-RW group had RW access, there was an overall group effect on total chow intake ($F(1, 120) = 24.67$ $p<0.001$) whereby the SED-RW group ate significant more chow. There was also a significant time*group interaction ($F(2, 120) = 30.49$ $p<0.001$) whereby at week four the RW-SED group was still eating more chow, but weeks five through six, when the SED-RW group had completely habituated to the running wheels, the SED-RW group began eating significantly more chow than the RW-SED group.

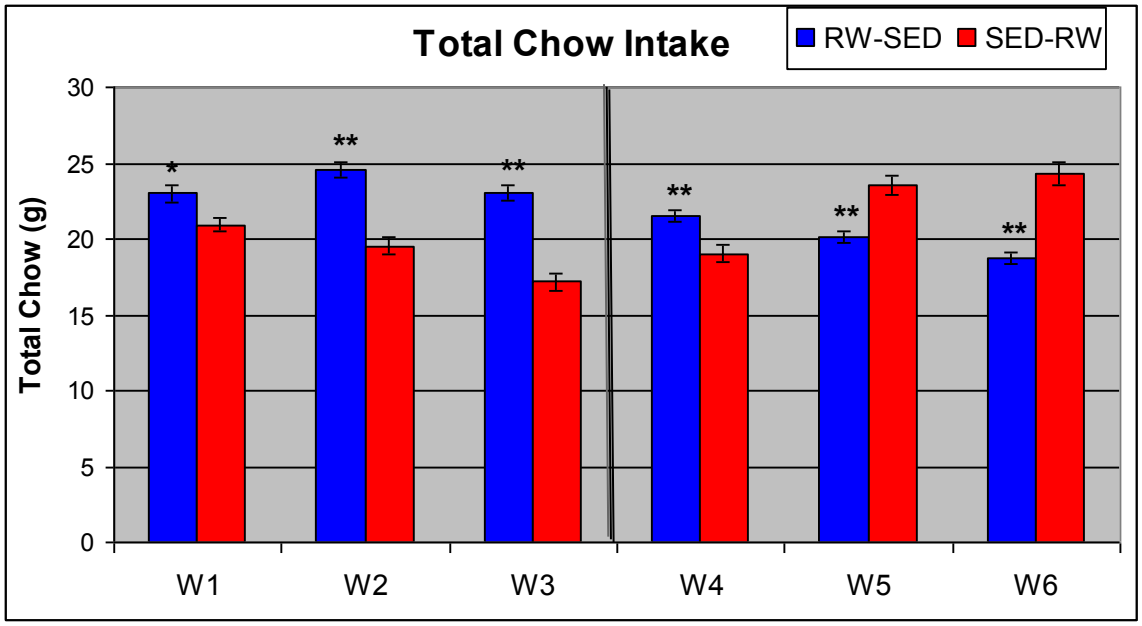

Figure 18. Average total chow intake of the RW-SED and SED-RW groups across weeks one through six. During weeks one through four, the RW-SED group (RW access) ate significantly more chow than the SED-RW group (sedentary). During weeks five and six, the RW-SED group (sedentary) ate significantly less chow than the SED-RW group (RW access). (*$p<0.01$, **$p\leq0.001$)

Over the entire six weeks, there was an overall group effect on total caloric intake (chow + Crisco) ($F(1, 240) = 35.717$ $p<0.001$), whereby the RW-SED group ate significantly more than the SED-RW group (Fig 19). There was also a significant time*group interaction ($F(5, 240) = 8.52$ $p<0.001$), that was primarily due to the significant decrease in Crisco intake by the SED-RW group on week 4 upon initial exposure to the running wheels (Fig 19). During the first three weeks there was a significant group effect on total intake ($F(1, 120) = 5.92$ $p=0.016$), whereby the RW-SED group ate more overall relative to the SED-RW group. There was also a significant time*group interaction ($F(2, 120) = 3.76$ $p=0.026$), whereby

during weeks one and two there was no significant difference in total intake but by week three the SED-RW group was eating less than the RW-SED group. During the last three weeks there was an overall group effect on total intake ($F(1, 120) = 35.83$ $p<0.001$) in which the RW-SED group ate significantly more overall, although this may be partially due to the significant decrease in Crisco intake by the SED-RW group on week 4 upon initial exposure to the running wheels. There was also a significant time*group interaction ($F(2, 120) = 14.05$ $p<0.001$), again due to the rebound hyperphagia of the SED-RW group after the first week of RW access on week 4.

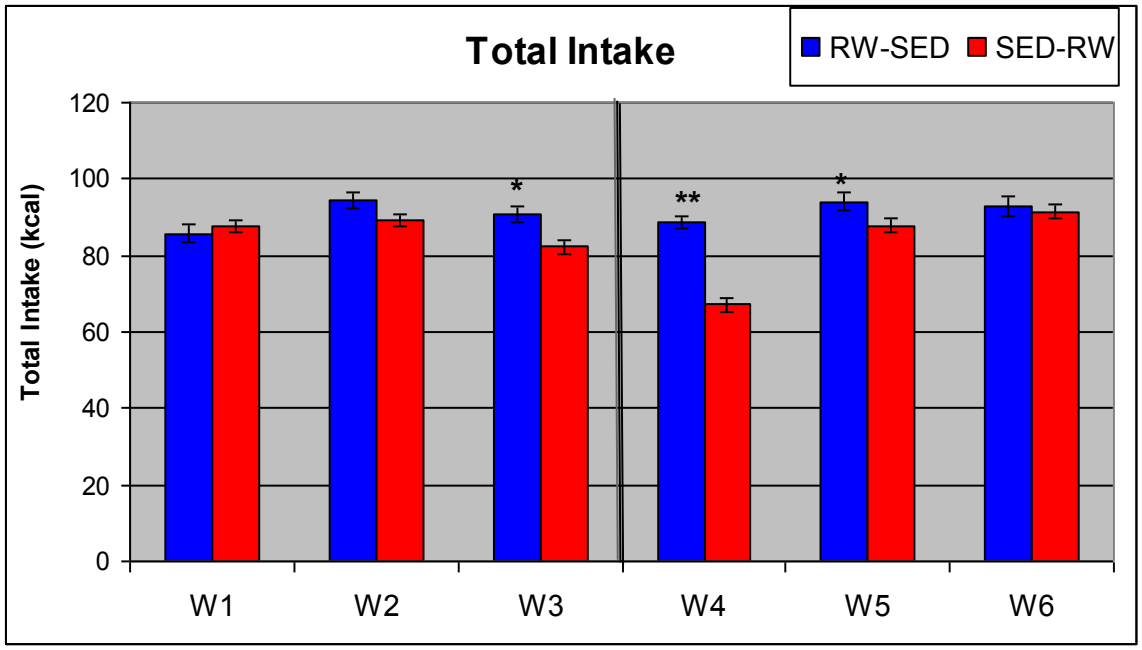

Figure 19. Average total caloric intake of the RW-SED and SED-RW groups across weeks one through six. During weeks three through five, the RW-SED group ate significantly more (chow + Crisco) than the SED-RW group. (*$p<0.05$, **$p\leq0.0001$)

During the binge paradigm there was no overall group effect on total running wheel activity (RWA) ($F(1, 36) = 3.99$ $p=0.053$), although there was a trend for the RW-SED group to run more than the SED-RW group (Fig 20). However, this could be due to the fact that the RW-SED group was able to habituate to the running wheels for five days before the binge protocol began, while the SED-RW group only had two full days to habituate to the wheels. There was no significant time*group interaction ($F(17,612) = 0.79$ $p=0.708$).

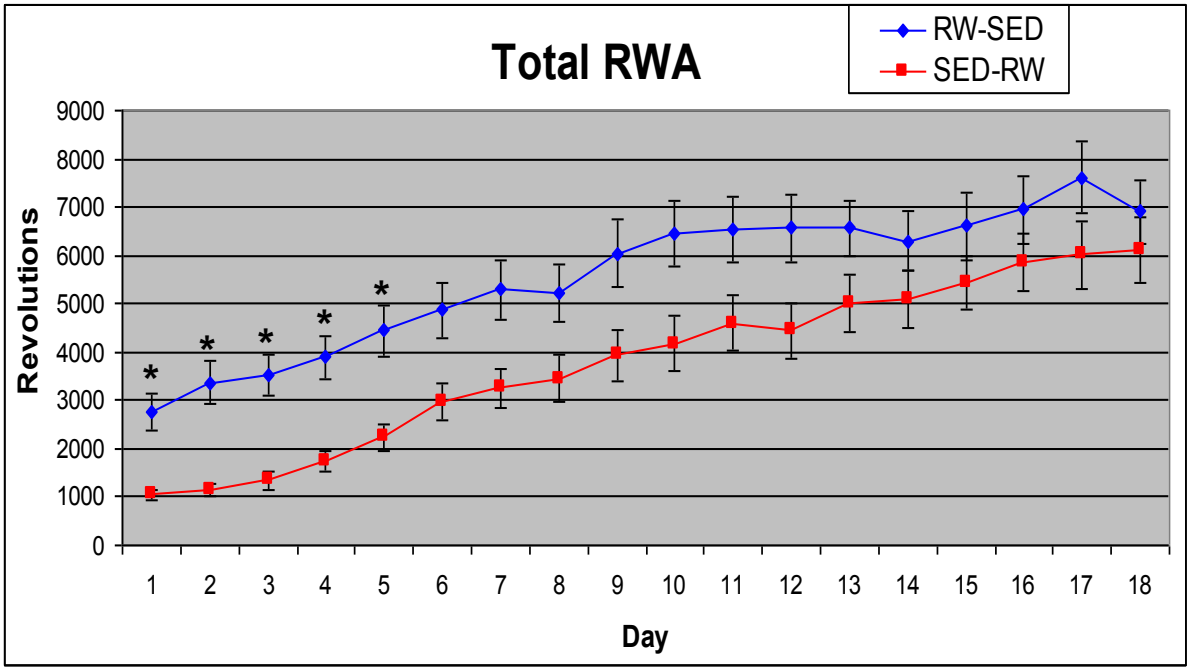

Figure 20. The graph shows the average total daily running wheel activity (RWA) for the RW-SED group during the first three weeks of the study when they had RW access and the total daily RWA for the SED-RW group during the last three weeks of the study when they had RW access. The RW-SED group ran significantly more than the SED-RW group during the first five days of RW access. (*$p<0.05$)

There was an overall group effect on RWA during the dark cycle ($F(1, 36) = 4.14$ $p=0.049$), whereby the rats in the RW-SED group ran significantly more than the rats in the SED-RW group. There was no significant time*group interaction ($F(17,612) = 1.07$ $p=0.379$). There was no group effect on RWA during the light cycle ($F(1, 36) = 1.21$ $p=0.279$), but there was a significant time*group interaction ($F(17,612) = 5.33$ $p<0.0001$), whereby after about a week and a half of RW access the SED-RW group began to run as much as the RW-SED group after they had habituated to the running wheels completely. There was also no group effect on food-anticipatory activity (FAA) ($F(1, 36) = 0.08$ $p=0.78$), but there was a significant time*group interaction ($F(17,612) = 9.20$ $p<0.0001$), whereby over time the SED-RW group began running more than the RW-SED group. There was also no group effect on RWA during the one hour Crisco access period ($F(1, 36) = 1.12$ $p=0.29$), but there was a significant time*group interaction ($F(17,612) = 4.34$ $p<0.0001$), whereby incrementally over time the SED-RW group began running more and the RW-SED group began leveling off their activity.

Plasma leptin levels

There was an overall group effect on the plasma leptin levels ($F(2,33)= 25.63$, $p<0.0001$). Plasma leptin levels at sacrifice were significantly higher in the RW-SED group compared to the SED-RW group ($p=0.00012$) and the control group ($p=0.00019$) (Fig 21).

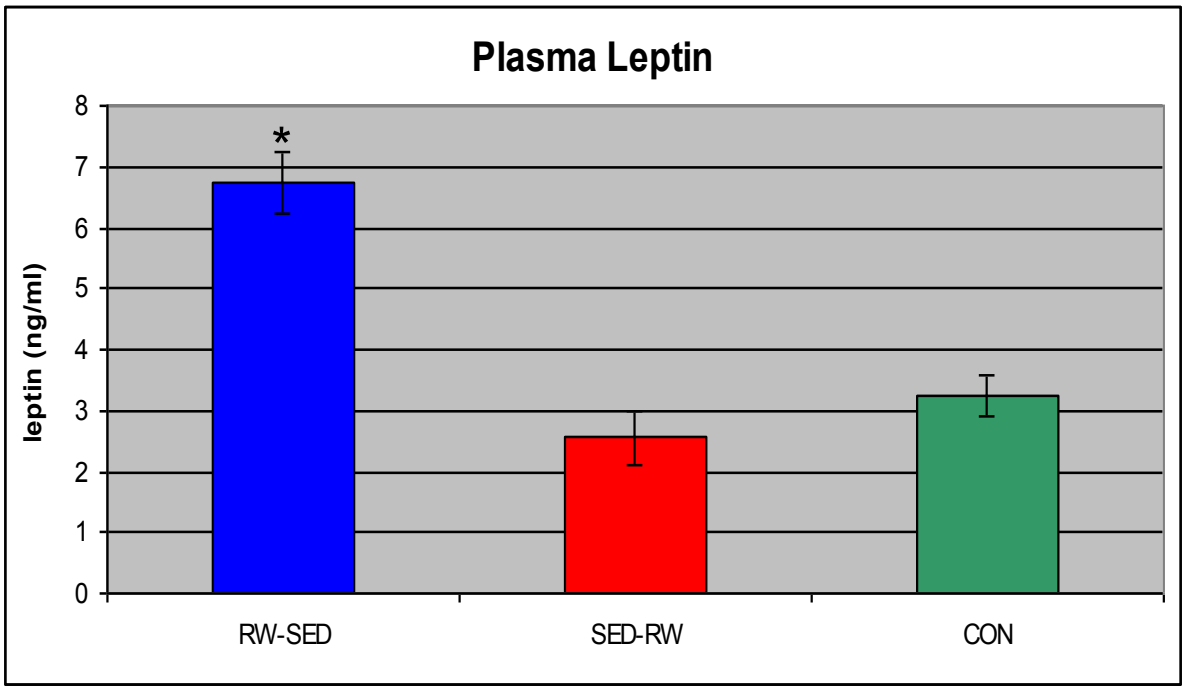

Figure 21. Plasma leptin levels in the RW-SED, SED-RW, and CON groups. The plasma leptin levels of the RW-SED group are significantly increased in comparison to both the SED-RW ($p=0.00012$) and CON groups ($p=0.00019$). (*$p<0.001$)

mRNA expression in the Ventral Tegmental area

In the ventral tegmental area there were no significant differences among the three groups (RW-SED, SED-RW, or SED CON) in expression of tyrosine hydroxylase (TH) (Table 3). However, there was a significant effect on the expression of the dopamine 2 receptor (D2R) ($F(2,25) = 5.8389$ $p=0.0083$). D2R was significantly decreased in the RW-SED group ($p=0.0076$) and the SED-RW group ($p=0.047$) in comparison to the CON group (Table 3).

Table 3. Dopaminergic mRNA gene expression in the ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC) of the RW-SED, SED-RW, and CON rats. The D2R expression in the VTA is significantly lower in the RW-SED (p=0.0076) and SED-RW (p=0.047) groups in comparison to the CON group. The D2R expression in the NAc is significantly lower in the SED-RW group in comparison to the CON group (p=0.024).

Group	RW-SED	SED-RW	CON
TH in VTA	0.87 +/- 0.13	0.88 +/- 0.09	1.2 +/- 0.32
D2R in VTA	**0.63* +/- 0.06**	**0.74* +/- 0.05**	1.06 +/- 0.15
D2R in NAc	0.96 +/- 0.03	**0.84* +/- 0.05**	1.01 +/- 0.04
D2R in PFC	1.04 +/- 0.07	1.06 +/- 0.09	1.01 +/- 0.06

mRNA expression in the Prefrontal Cortex

In the prefrontal cortex there were no significant differences in expression of the D2R (Table 3) or of the mu-opioid receptor (Oprm1) (Table 4) among the three groups (RW-SED, SED-RW, or CON).

mRNA expression in the Nucleus Accumbens

In the nucleus accumbens there was a significant effect on the expression of the D2R ($F(2,26) = 4.66$ $p=0.019$). Expression in the SED-RW group was reduced in relation to levels in the CON group (p=0.024) and there was a trend for decreased expression relative to the RW-SED group (Table 3). There was also a significant effect on the expression of the Oprm1 ($F(2,27) = 5.42$ $p=0.011$). Expression in the RW-SED group was elevated in comparison to the level of the CON group (p=0.0076) (Table 4). There was a trend for an increase in expression of the Oprm1 in the SED-RW group (p=0.058), relative to the CON group.

Table 4. Opioid mRNA gene expression in the ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC) of the RW-SED, SED-RW, and CON rats. The Oprm1 expression in the NAc is significantly higher in the RW-SED group in comparison to the CON group (p=0.0076).

Group	RW-SED	SED-RW	CON
Oprm1 in NAc	**1.47* +/- 0.09**	1.28 +/- 0.08	1.03 +/- 0.1
Oprm1 in PFC	0.97 +/- 0.03	0.95 +/- 0.06	1.01 +/- 0.05

mRNA expression in the Arcuate Nucleus

In the arcuate nucleus there was a significant effect on expression of the Agouti-related peptide (AgRP) ($F(2,26) = 10.62$ $p=0.0004$). Expression levels in the SED-RW group were significantly elevated relative to levels of the CON group ($p=0.00086$) and the RW-SED group ($p=0.0041$) (Table 5). There was also a significant effect on expression of Pro-opiomelanocortin (POMC) ($F(2,24) = 18.76$ $p<0.001$). Expression levels were elevated in the RW-SED group in comparison to levels in both the SED-RW group ($p=0.0008$) and the CON group ($p=0.0001$) (Table 5). There was also a significant effect on levels of expression of Neuropeptide Y (NPY) ($F(2,24) = 17.86$ $p<0.001$). Expression levels were elevated in the SED-RW group in comparison to levels in both the RW-SED group ($p=0.00057$) and the CON group ($p=0.0002$) (Table 5). Finally, there was a significant effect on levels of expression of the leptin receptor (LepR) ($F(2,27) = 3.86$ $p=0.03$). Expression levels were significantly decreased in the SED-RW group ($p=0.039$), relative to the levels in CON group, and there was a trend for a decrease in the expression of the RW-SED group, relative to the CON group (Table 5).

mRNA expression in the Lateral Hypothalamus

In the lateral hypothalamus there were no significant differences in expression of orexin among the three groups (RW-SED, SED-RW, or CON). There were also no significant differences in expression of the LepR among the three groups (RW-SED, SED-RW, or CON) (Table 5).

Table 5. Neuropeptide changes in the arcuate nucleus (ARC) and lateral hypothalamus (LH) of the RW-SED, SED-RW, and CON rats. The AgRP expression in the ARC is significantly higher in the SED-RW group in comparison to both the RW-SED (p=0.0041) and CON groups (p=0.00086). The POMC expression in the ARC is significantly elevated in the RW-SED group in comparison to both the SED-RW group (p=0.0008) and the CON group (p=0.0001). The NPY expression in the ARC is significantly higher in the SED-RW group in comparison to both the RW-SED (p=0.00057) and CON groups (p=0.0002). The LepR expression in the ARC is significantly lower in the SED-RW group in comparison to the CON group (p=0.039), and there was a trend for a decrease in the expression of the RW-SED group, relative to the CON group.

Group	RW-SED	SED-RW	CON
AgRP in ARC	1.2 +/- 0.08	**1.88* +/- 0.19**	1.03 +/- 0.09
NPY in ARC	1.29 +/- 0.09	**2.25* +/- 0.22**	1.01 +/- 0.06
POMC in ARC	**2.12* +/- 0.14**	1.43 +/- 0.09	1.06 +/- 0.14
LepR in ARC	0.84 +/- 0.05	**0.81* +/- 0.04**	1.02 +/- 0.07
LepR in LH	1.22 +/- 0.14	1.06 +/- 0.1	1.01 +/- 0.06

Discussion:

The aim of this study was to investigate how voluntary running wheel activity affects binge behavior within this limited access rat model of binge eating. Our data show that there was an overall effect of the running wheel (RW) on Crisco intake. During the first three weeks of the experiment when the RW-SED group had access to running wheels (RWs), they consumed significantly less Crisco than the SED-RW group who was sedentary. However, once running wheel access was reversed and the SED-RW group had access to the RWs, they ate significantly less Crisco than the RW-SED group that was now sedentary. Thus, while prior experience with a RW did not prevent bingeing once running wheel activity (RWA) had ceased, established bingeing behavior did subside and significantly decrease when the rats were given free access to a RW.

During the first half of the experiment, the RW-SED group was initially consuming significantly less Crisco than the currently sedentary SED-RW group. However, once the RW access was switched, intake escalated in the newly sedentary RW-SED group, ultimately reaching significantly higher Crisco intakes than the SED-RW group, who now had access to a RW. In experiments by Satvat et al., it has been shown that rats introduced to a sucrose solution and running wheel simultaneously engage in running wheel activity but avoid consuming the sweet solution (84). Additionally, as shown by Liang et al., rats that had developed persistent preferences to a solid palatable food (high fat diet (HFD)) and consumed significantly more HFD than the high carbohydrate chow diet (93) reduced their intakes of the HFD as soon as running wheel access was allowed, and thus decreased their preference of a previously preferred HFD in a two-diet (standard chow vs. HFD) choice

feeding schedule (91, 92). Thus, while the paradigm used here did not involve daily access to a HFD but rather intermittent access, the data presented here mirrors the data previously described, whereby access to a RW can significantly decrease consumption of a highly palatable food.

During the first three weeks of the experiment, the RW-SED group weighed significantly less than the sedentary SED-RW group. However, once the RW access was switched, by week 6 the SED-RW group weighed significantly less than the sedentary RW-SED group. It has been shown that running suppresses weight gain even with HF diet available (91, 214, 215). The suppression of weight gain is not simply due to reduced intakes of chow and high fat diet in wheel running (WR) rats compared to Sedentary rats with similar diet availability because sedentary paired-fed rats that consume equal amounts of chow and high fat diets also gain significantly more weight than rats with running wheel access. Thus, we can see that not only does running wheel activity help to decrease bingeing behavior, but it can help to decrease body weight, which is a beneficial outcome for individuals with binge eating because not only does it help to improve overall health but it can help improve self-image and body satisfaction, which is often very low in these individuals (183, 184, 212).

During the first four weeks of the study, the RW-SED group ate significantly more chow than the SED-RW group. However, by week 5, a week into the SED-RW group's access to RWs, they began eating significantly more chow than the newly sedentary RW-SED group. Thus, access to a RW seems to alter their food preference, whereby they prefer standard rodent chow over high fat Crisco. This is in line with recent research by Liang et al.,

showing that in male rats, when running wheel access and a novel palatable high fat diet were provided simultaneously, the rats preferred the chow diet, demonstrating persistent avoidance to the palatable diet (93). Additionally, all of the rats displayed a sawtooth pattern of chow intake, whereby they ate more chow on days without Crisco access and less chow on days with Crisco access as a way of compensating for the extra calories from Crisco. In studies by Corwin et al. where they also give rats intermittent, limited access to vegetable shortening, they also report seeing this binge/compensate pattern of feeding whereby rats overeat on days that palatable food is provided and undereat when palatable food is not provided (101, 121, 124, 126). Additionally, in studies by Avena, Hoebel and colleagues in which rats have intermittent access to a sugar solution, rats are able to regulate their caloric intake as well and compensate for the excess calories acquired from sugar by eating less chow (136).

Several changes in gene expression were found within brain regions involved with appetite and energy balance. Within the arcuate nucleus (ARC), we found a significant increase in expression of the Agouti-related peptide (AgRP) in the SED-RW group, in comparison to the RW-SED and CON group. Due to the fact that AgRP works by increasing appetite and decreasing metabolism and energy expenditure, it is understandable that the SED-RW group has increased expression as they were consuming less Crisco than the RW-SED group and was the only group exercising. There was also a significant increase in expression of Neuropeptide Y (NPY) in the SED-RW group, in comparison to the RW-SED and CON groups. NPY is co-expressed with AgRP and, like AgRP, works to increase food intake and the storage of energy as fat, and the expression of these often mirror each

other. It has been shown that NPY preferentially stimulates the consumption of a carbohydrate-rich diet and, in turn, is found to be reduced in fat-preferring rats (186, 216). This is in line with our data, which shows the SED-RW group, who are now consuming significantly less Crisco and significantly more of the carbohydrate-rich rodent chow, expressing the highest levels of NPY, while the RW-SED group, who are now sedentary and bingeing, have significantly reduced NPY levels in comparison.

Additionally, within the arcuate nucleus, there was a significant increase in the expression of Pro-opiomelanocortin (POMC) in the RW-SED group in comparison to both the SED-RW group and the CON group. The expression of POMC in the SED-RW group was also increased in comparison to the CON group. Alpha-MSH, a product of POMC, is a satiety peptide and works to decrease appetite, and thus the increased expression could be a response to the higher binge intake of Crisco of the RW-SED group at the time of sacrifice. The SED-RW group, while not consuming as much Crisco as the RW-SED group, is still consuming Crisco and thus that might explain why they have greater expression in comparison to the CON group.

Lastly, there was a significant decrease in expression of the leptin receptor (LepR) in the SED-RW group and a trend for decreased expression in the RW-SED group, relative to the CON group. Consequently, we looked at their plasma leptin levels. Within the RW-SED group, we found increased leptin levels at sacrifice compared to the CON group. This is plausible because the RW-SED rats are sedentary and bingeing at this time and weigh significantly more than the SED-RW group. Furthermore, leptin is a satiety hormone made

by adipose cells and released in proportion to body adiposity, and it contributes to the regulation of energy balance by inhibiting hunger. Thus, these increased leptin levels could in turn contribute to a down regulation of the leptin receptor. However, the SED-RW group did not have increased leptin levels at sacrifice compared to the CON group. They may have previously had increased leptin levels (similar to the RW-SED group), and down regulated leptin receptors, during the first three weeks of the study when they were sedentary, but the current receptor expression may not adequately reflect the newly lower leptin levels; it may take more time for the expression of the receptors to adjust. This effect is in contrast to levels of the LepR in the lateral hypothalamus (LH), which showed no differences in expression among the three groups, and thus this effect on the LepR expression appears to be targeted to the ARC.

Binge behavior and running wheel access also resulted in several changes in gene expression within brain reward areas. Overall, we did not find any significant effects on gene expression of the mu-opioid receptor (Oprm1) or the dopamine 2 receptor (D2R) in the prefrontal cortex (PFC). However, in the nucleus accumbens (NAc), there was a significant decrease in the expression of the D2R in the SED-RW group in comparison to the CON group, and a trend for decreased expression relative to the RW-SED group. In a study by Greenwood et al., it was demonstrated that six weeks of voluntary wheel running resulted in decreased D2R mRNA levels in the NAc of rodents (178). Thus, the SED-RW group may have decreased expression due to their current running wheel activity. In comparison to data presented by other groups utilizing a binge-eating paradigm, most report decreased D2R expression in the NAc of rats that are currently bingeing (134, 140, 213). However, our data

show decreased expression only in the rats that have significantly decreased their Crisco consumption and are now running. Thus, there is the possibility that it takes more time for the D2R expression to adjust to the bingeing behavior, and consequently the expression in the RW-SED group has not yet decreased.

Additionally, within the NAc, there was a significant increase in the expression of the Oprm1 in the RW-SED group in comparison to the CON group. There was also a trend for an increase in expression of the Oprm1 in the SED-RW group, relative to the CON group. Data generated from the sugar addiction model has shown that sugar bingeing also significantly enhances mu-opioid receptor binding in the NAc shell compared with chow-fed controls (124, 140). Additionally, the fact that sugar-bingeing rats are sensitive to the effects of the opioid antagonist naloxone, which can precipitate signs of withdrawal (124, 145), suggests that repeated bouts of excessive sugar intake can alter brain opioid systems. Moreover, results from a model that employs a history of dieting (HD) + stress, and uses Oreo cookies as the highly palatable diet, also lend support for the role of opioids in binge eating behavior. HD + stress-induced binge eating is abolished by naloxone, a mixed kappa/mu opioid receptor antagonist (105). Consistent with opioid-receptor sensitivity, the opioid-receptor agonist butorphanol achieves a more potent hyperphagia in the binge-eating rats relative to the control groups despite their already increased levels of intake (105). Additionally, findings from Corwin's limited access model, which uses vegetable shortening as the highly palatable diet, also present evidence of opioids serving a role in binge eating behavior. In this limited access model, the opioid antagonist naltrexone reduces intake of solid 100% fat (shortening), solid emulsions made with different concentrations of shortening (32%, 56%), and fat

sucrose mixtures in rats with intermittent limited access to the palatable food (124, 119). Thus, regardless of the model or type of highly palatable food used, it seems that the endogenous opioids play a role in and are altered by bingeing behavior.

Within the ventral tegmental area (VTA), there was a significant decrease in the D2R expression in both the RW-SED and SED-RW groups, relative to the CON group. The D2R acts as an autoreceptor within the VTA, meaning that it works as a part of the negative feedback loop to decrease dopamine release. Thus, decreased expression of this dopamine receptor implies less inhibition on dopamine release, which could result in stimulation of the reward pathway. There was also a trend for a decrease in tyrosine hydroxylase (TH) in both the RW-SED and SED-RW groups, relative to the CON group, but with the amount of variability, this did not reach statistical significance.

Overall, it appears as though expression of energy balance genes is changed in the appropriate directions relative to the patterns of Crisco intake. Rats that are currently bingeing (RW-SED) show decreased expression of orexigenic peptides (AgRP and NPY) but increased expression of the anorexigenic peptide precursor (POMC). However, these changes are not sufficient to alter behavior as rats continue to binge. Thus, changes in the reward pathways of the brain may be overriding these changes in energy balance pathways, as we see indications of the reward pathways being stimulated in these bingeing animals. Additionally, it has been shown that not only is consuming a palatable high-fat diet rewarding (70, 124, 138, 167, 177), but running wheel activity is rewarding for rodents as well (74, 178), which could explain why both groups of rats demonstrate stimulation of brain

reward pathways; the RW-SED group could be receiving a rewarding effect from bingeing on Crisco, and the SED-RW group could be receiving a rewarding effect from wheel running.

As a whole, there are various therapeutic implications that can be taken from this research. Although obesity is not a criterion for BED, there is a strong association between the two (202, 203). More than 65% of BED patients are obese (204) and within a group of patients seeking treatment for obesity, the prevalence of BED is approximately 25% (205). Obese binge eaters often show more severe obesity and greater eating disorder psychopathology, i.e. more weight and shape concerns and body dissatisfaction, more emotional eating, more negative self-evaluations and lower self-esteem compared with obese non-binge eaters (205, 206). Physical consequences of BED are largely due to co-morbid obesity and a sedentary lifestyle. There are a few studies (183, 184, 207, 208) that have considered physical activity levels among BED patients. The level of physical activity reported by obese individuals who binge eat in these studies is roughly less than half of the levels reported by community samples using similar assessment methods (209). Because of severe co-morbid psychiatric and physical conditions, BED has been characterized as one of the most difficult psychiatric conditions to treat (1,2). Specialized psychotherapies, such as cognitive behavioral therapy (CBT), are somewhat effective at reducing binge eating, but not all BED patients respond adequately (204, 210, 184). Additionally, research into the pharmacotherapy of BED is only in its early stages, and only recently has one medication (Vyvanse) received approval from a regulatory body for use (211).

Since weight and shape concern, body dissatisfaction, obesity, and physical inactivity are central to BED, and patients perceive improving self-esteem and mood as core elements in their treatment (212), physical activity might be an effective supplementary treatment. Two randomized control trials (183, 184) indicate that patients who follow an aerobic exercise program report fewer binges per week. In a study by Levine et al. (183), more than 80% of the patients following a 6-month walking program were abstinent from binge eating. In a study by Pendleton et al. (184), a combination of aerobic exercise and CBT resulted in significantly fewer binges per week than CBT alone. The findings from the current study demonstrate the same basic phenomenon, whereby general running wheel activity helps to significantly decrease binge eating in rats. Thus, moderate physical activity could be a helpful supplementary treatment, as it not only seems to help decrease binge episodes, but also helps decrease body weight a bit, and could additionally help improve body satisfaction and self-esteem. However, one must be aware that too much physical activity can potentially be dangerous and addictive, as is the case with many patients with Anorexia nervosa, and in rodents exposed to the activity-based anorexia paradigm (6,7, 10, 14). Overall, a regular routine of moderate physical activity would be beneficial with the treatment of BED, helping to decrease binges and increase mood and body image.

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222. Levine AS, Billington CJ. Why do we eat? A neural systems approach. Annu. Rev. Nutr., 1997; 17: 597–619.

Curriculum Vitae

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Educational History:

Johns Hopkins University School of Medicine
- PhD, Cellular and Molecular Medicine Program
- Graduation Date: July 2015
- Laboratory of Dr. Timothy Moran
- Department of Psychiatry and Behavioral Sciences
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Denison University
- Bachelor of Science (B.S.) in Biology Degree
- Graduation Date: May 2010
- Biology Major, Psychology Minor, Neuroscience Concentration

Other Professional Experience:

- Research Rotation: 2010, Lab of Dr. Christopher Ross, Johns Hopkins University School of Medicine
- Research Rotation: 2011, Lab of Dr. Wenzhen Duan, Johns Hopkins University School of Medicine

Awards:

--2014 Early Career Investigator Award (October 2014)-- The Obesity Society (TOS), grant in the amount of $1000.00, from Francesca Dea (Executive Director)

Teaching:

Instructor for Duke-TIP (Talent Identification Program)
- Summer 2013, June (Term 1) and July (Term 2)
- Taught "The Brain, Intelligence, and Creativity" class at Davidson Campus
- Taught college level course material to gifted seventh and eighth grade students from across the country
- Material included Introductory Neuroscience, Neuropharmacology, Introductory Psychology, and Social Psychology

Memberships:

- Member of Society for the Study of Ingestive Behavior (SSIB): March 2013-present
- Member of The Obesity Society (TOS): July 2014-present

Talks/Posters:

Albertz, Jennifer, Moran, Timothy. *The Effects of Voluntary Exercise on a Rat Model of Binge Eating* (poster). Johns Hopkins University Psychiatry and Behavioral Sciences annual Potpourri, Baltimore, MD, May 2014.

Albertz, Jennifer, Moran, Timothy. *The Effects of Voluntary Exercise on a Rat Model of Binge Eating and its Consequences* (poster). The Society for the Study of Ingestive Behavior (SSIB) annual meeting, Seattle, Washington, July 29-August 2, 2014.

Albertz, Jennifer, Boersma, Gretha, Tamashiro, Kellie, and Moran, Timothy. *The Effects of Prenatal Stress and Coping Style on Binge Behavior* (poster). The Obesity Society (TOS) annual meeting, Boston, Massachusetts, November 2014.

Albertz, Jennifer, Moran, Timothy. (2015) *The Effects of Voluntary Exercise on a Rat Model of Binge Eating and its Consequences* (talk). Johns Hopkins University Psychiatry and Behavioral Sciences annual Potpourri, Baltimore, MD, May 2015.

Publications:

Jankord, Ryan, Zhang, Rong, Flak, Jonathan N., Solomon, Matia B., **Albertz**, **Jennifer** and Herman, James P. (2010) Stress activation of IL-6 neurons in the hypothalamus. *Am J Physiol Regulatory Integrative Comp Physiol* 299(1):343- 351.

Jankord, Ryan, Solomon, Matia B., **Albertz**, **Jennifer,** Flak, Jonathan N., Zhang, Rong, and Herman, James P. (2011) Stress vulnerability during adolescent development in rats. *Endocrinology* 152(2):629–638.

Jin, Jing, **Albertz**, **Jennifer,** Guo, Zhihong, Peng, Qi, Rudow, Gay, Troncoso, Juan C., Ross, Christopher A., and Duan, Wenzhen. (2013) Neuroprotective effects of PPAR-γ agonist rosiglitazone in N171-82Q mouse model of Huntington's disease. *J. Neurochem* 125(3) 410-419.

Boersma, Gretha J., **Albertz**, **Jennifer,** Moody, Laura A., Liang, Nu-Chu, Aryal, S Moran, Timothy H, and Tamashiro, Kellie L. (2015) Prenatally-stressed passive-coping rats have increased weight-loss and altered Agrp and Hrct expression after Activity-based anorexia. *In review.*

Service:

- Incentive Mentoring Program (IMP) (2010-2011), a program that extends a school-based tutoring program to the home, providing both academic and social support to youth at a local high school who are struggling with poverty, drugs, and violence.